BIOPESTICIDES REGISTRATION ACTION DOCUMENT

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)

PC Code: 006599

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division

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I. Overview

Notes: The Environmental Protection Agency's human health and environmental assessment summaries often refer to Vip3Aa proteins, which include the Vip3Aa20 insecticidal protein as expressed in corn. Additionally, throughout part I of this Biopesticides Registration Action Document (BRAD), maize and corn are used interchangeably but hold the same meaning.

A. Executive 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. EPA has also conditionally registered two other products, *Bt*11 x MIR162 corn (expressing previously registered Cry1Ab and Vip3Aa20, respectively) and *Bt*11 x MIR162 x MIR604 corn (expressing previously registered Cry1Ab, Vip3Aa20, and previously registered mCry3A, respectively), that contain the new active ingredient and are intended for commercial distribution and use. The Agency has determined that the use of these pesticides are in the public interest and that they will not cause any unreasonable adverse effects on the environment during the time of conditional registration. The registrant for all of these products is Syngenta.

The new plant-incorporated protectant products produce their own insecticidal proteins within the corn plant. These insecticidal proteins were derived from *Bacillus thuringiensis* (*Bt*), a naturally occurring soil bacterium. The Vip3Aa20 insecticidal protein expressed in all three of the products controls certain lepidopteran pests of corn, the Cry1Ab insecticidal protein expressed in two of the products also controls certain lepidopteran pests of corn (in particular, European corn borer), and the mCry3A insecticidal protein expressed in only one product controls coleopteran 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.

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, *Bt*11 x MIR162 corn, and *Bt*11 x MIR162 x MIR604 corn 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 these products, as well as for other corn PIP products. Furthermore, as additional registered *Bt* corn products, MIR162 maize, *Bt*11 x MIR162 corn, and *Bt*11 x MIR162 x MIR604 corn will likely result in direct and indirect human and environmental health benefits by providing growers with alternative *Bt* corn options 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, *Bt*11 x MIR162 corn, and *Bt*11 x MIR162 x MIR604 corn may also result in further reduction of chemical insecticide use by growers.

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 and its stacked and/or pyramided products (*Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn) will be in the public interest. Utilization of these products will result in direct and indirect human and environmental health benefits by providing growers with additional *Bt* corn products 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.

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 were 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.

DNA characterization (i.e., Southern blot analysis) was used to confirm the presence of the *cry1Ab* and *pat* genes from the parental Event *Bt*11 and the *vip3Aa20* and *pmi* genes from the parental Event MIR162 in *Bt*11 x MIR162 corn. Samples from *Bt*11 x MIR162 corn gave the same results as those observed for the individual parental events, indicating that the molecular characterization data provided for the individual parental events are also applicable to *Bt*11 x MIR162 corn.

Southern blot analysis was also used to confirm the presence of the *cry1Ab* and *pat* genes from the parental Event *Bt*11, *vip3Aa20* and *pmi* genes from the parental Event MIR162, and *mcry3A* and *pmi* genes from the parental Event MIR604 in *Bt*11 x MIR162 x MIR604 corn. Samples from *Bt*11 x MIR162 x MIR604 corn gave the same results as those observed for the individual parental events, indicating that the molecular characterization data provided for the individual parental events are also applicable to *Bt*11 x MIR162 x MIR604 corn.

Protein expression data, together with data indicating that the insecticidal proteins in Events *Bt*11, MIR162, and MIR604 act individually to effect a typical midgut pathology in susceptible insects like previously studied *Bt* delta-endotoxins, demonstrate that no synergistic action or interaction of these insecticidal proteins is known or expected to occur. Thus, the data on the individual events and individual insecticidal proteins can be used to support the safety of the stacked and/or pyramided products, *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn.

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 LD_{50} 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.

With respect to the previously registered plant-incorporated protectants that have been combined with Vip3Aa20 to create *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn, the following web links provide access to Biopesticides Registration Action Documents that contain comprehensive human health assessments for each active ingredient:

- Event *Bt*11 (Cry1Ab) corn: *http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm*
- Event MIR604 (mCry3A) corn: *http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006509.pdf*

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.

Prior environmental assessments for the Cry1Ab and mCry3A insecticidal proteins can be found at the web links provided in the "Human Health Assessment" section above.

The synergism studies, non-target organism toxicity testing, and field studies reviewed for the *Bt*11, MIR162, and MIR604 parental events indicate their associated combined-PIP products, *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn, will not result in any unexpected interaction related to an antagonistic or synergistic action to target and non-target insects. Therefore, it is extremely unlikely that the Vip3Aa, Cry1Ab, and mCry3A insecticidal proteins contained in a single plant will impart any hazard to non-target organisms exposed to the *Bt*11 x MIR162 and *Bt*11 x MIR162 x MIR604 corn hybrids in the environment.

Furthermore, the compilation of ecotoxicity studies on non-target organisms, evaluation for synergism between the test proteins, efficacy data, and field data support the bridging of the

environmental risk assessment from the parental events to the combined-PIP products. Based on prior assessments conducted on the Vip3Aa, Cry1Ab, and mCry3A insecticidal proteins individually, the environmental risk assessment for the *Bt*11 x MIR162 and *Bt*11 x MIR162 x MIR604 corn hybrids indicates that no unreasonable harm will result to the environment or any Federally listed endangered or threatened species from commercial cultivation of these corn hybrids.

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.

The other two conditionally registered products, *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn, require insect resistance management programs because they are intended for unlimited commercial distribution. In order to reduce the possibility of the target pests developing resistance to the Cry1Ab and Vip3Aa20 insecticidal proteins expressed in *Bt*11 x MIR162 corn and the Cry1Ab, Vip3Aa20, and mCry3A insecticidal proteins expressed in *Bt*11 x MIR162 x MIR604 corn, EPA is requiring Syngenta to ensure that a portion of the planted acreage of each of these products be set aside where non-*Bt* corn, non-lepidopteran-resistant *Bt* corn, and/or non-corn rootworm-protected *Bt* corn (depending on the product and refuge option employed) will be grown to serve as a "refuge." The refuge options for each stacked and/or pyramided product are described below.

i) Bt11 x MIR162 Corn (Lepidopteran Active)

- Specifically, growers must plant a structured refuge of at least 20% non-*Bt* corn and/or non-lepidopteran-resistant *Bt* corn that may be treated with insecticides, as detailed below, to control lepidopteran stalk-boring and other pests.
- Refuge planting options include: separate fields, blocks within fields (e.g., along the edges or headlands), perimeter strips, and strips across the field.
- External refuges must be planted within ½ mile.
- When planting the refuge as strips across the field or as perimeter strips, refuges must be at least four consecutive rows wide.

- Insecticide treatments for control of European corn borer (ECB), corn earworm (CEW), southwestern corn borer (SWCB), and other lepidopteran pests listed on the label, grower guides, or other educational material may be applied only if economic thresholds are reached for one or more of these target pests. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents or crop consultants). Instructions to growers will specify that microbial *Bt* insecticides must not be applied to non-*Bt* corn and/or non-lepidopteran-resistant *Bt* corn refuges.

ii) Bt11 x MIR162 x MIR604 Corn (Lepidopteran and Coleopteran Active)

Two options for deployment of the refuge are available to growers.

The first option is planting a common refuge for both corn borers and corn rootworms. The common refuge must be planted with corn hybrids that do not contain *Bt* technologies for the control of corn rootworms or corn borers. The refuge area must represent at least 20% of the grower's corn acres (i.e., sum of *Bt*11 x MIR162 x MIR604 corn acres and refuge acres). It must be planted as a block adjacent to the *Bt*11 x MIR162 x MIR604 corn field, perimeter strips, or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least four consecutive rows wide. If the common refuge is planted on rotated ground, then *Bt*11 x MIR162 x MIR604 corn must also be planted on rotated ground. If the common refuge is planted in continuous corn, the *Bt*11 x MIR162 x MIR604 corn field may be planted on either continuous or rotated land. The common refuge can be treated with a soil-applied or seed-applied insecticide to control rootworm larvae and other soil pests. The refuge can also be treated with a non-*Bt* foliar insecticide for control of late season pests, if pest pressure reaches an economic threshold for damage; however, if rootworm adults are present at the time of foliar applications, then the *Bt*11 x MIR162 x MIR604 corn field must be treated in a similar manner. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents or crop consultants). Pests other than adult corn rootworms can be treated with an appropriate pest-labeled insecticide on the common refuge acres without treating the *Bt*11 x MIR162 x MIR604 corn acres only if treatment occurs when adult corn rootworms are not present. Pests on the *Bt*11 x MIR162 x MIR604 corn acres can be treated as needed without having to treat the common refuge.

The second option is planting separate refuge areas for corn borers and corn rootworms. The corn borer refuge must be planted with a non-*Bt*/lepidopteran-protected hybrid, must represent at least 20% of the grower's corn acres (i.e., sum of *Bt*11 x MIR162 x MIR604 corn acres and corn borer refuge acres), and must be planted within ½ mile of the *Bt*11 x MIR162 x MIR604 corn field. Refuge planting options include separate fields, blocks within fields (e.g., along the edges or headlands), perimeter strips, or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least four consecutive rows wide. The corn borer

refuge can be treated with a soil-applied or seed-applied insecticide for corn rootworm larval control or a non-*Bt* foliar-applied insecticide for corn borer control, if pest pressure reaches an economic threshold for damage. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents or crop consultants).

The corn rootworm refuge must be planted with a non-*Bt*/corn rootworm-protected hybrid, but can be planted with *Bt* corn hybrids that control corn borers. The corn rootworm refuge must represent at least 20% of the grower's corn acres (i.e., sum of *Bt*11 x MIR162 x MIR604 corn acres and corn rootworm refuge acres) and must be planted as an adjacent block, perimeter strips, or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least four consecutive rows wide. If the rootworm refuge is planted on rotated ground, then *Bt*11 x MIR162 x MIR604 corn must also be planted on rotated ground. If the rootworm refuge is planted in continuous corn, the *Bt*11 x MIR162 x MIR604 corn field may be planted on either continuous or rotated land. More generally, the rootworm refuge should utilize comparable agronomic practices as the *Bt*11 x MIR162 x MIR604 corn acres. The corn rootworm refuge can be treated with a soil-applied or seed-applied insecticide to control rootworm larvae and other soil pests. The refuge can also be treated with a non-*Bt* foliar insecticide for control of late season pests; however, if rootworm adults are present at the time of foliar applications, then the *Bt*11 x MIR162 x MIR604 corn field must be treated in a similar manner. Pests other than adult corn rootworms can be treated on the rootworm refuge acres without treating the *Bt*11 x MIR162 x MIR604 corn acres only if treatment occurs when adult corn rootworms are not present or if a pesticide without activity against adult corn rootworms is used. Pests on the *Bt*11 x MIR162 x MIR604 corn acres can be treated as needed without having to treat the rootworm refuge.

BPPD has concluded that based on the modeling, dose, and efficacy studies, the refuge options, as described above, are acceptable for *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn. Syngenta will also be required to conduct a resistance monitoring program for Cry1Ab, Vip3Aa20, and mCry3A with the major target pests. Additional requirements for remedial action (in the event of resistance), grower agreements, grower education, compliance assurance, and annual reports have also been implemented for *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn as terms of registration.

B. Use Profile

Pesticide Names:

a) *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)

b) *Bacillus thuringiensis* Cry1Ab delta-endotoxin protein and the genetic material necessary for its production (*via* elements of vector pZO1502) in Event *Bt*11 corn (OECD Unique Identifier: SYN-BTØ11-1) x *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)

c) *Bacillus thuringiensis* Cry1Ab delta-endotoxin protein and the genetic material necessary for its production (*via* elements of vector pZO1502) in Event *Bt*11 corn (OECD Unique Identifier: SYN-BTØ11-1) x *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) x modified Cry3A protein and the genetic material necessary for its production (*via* elements of vector pZM26) in Event MIR604 corn (OECD Unique Identifier: SYN-IR6Ø4-5)

Trade and Other Names:

a) MIR162 Maize or MIR162 Corn
b) *Bt*11 x MIR162 Corn or Agrisure™ 2100
c) *Bt*11 x MIR162 x MIR604 Corn or Agrisure™ 3100

Office of Pesticide Programs (OPP) Chemical Codes:

a) 006599 (Vip3Aa20)
b) 006444(Cry1Ab) and 006599 (Vip3Aa20)
c) 006444(Cry1Ab), 006599 (Vip3Aa20), and 006509 (mCry3A)

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

Use: Field Corn

Target Pests:

a) 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*)

b) European corn borer (*Ostrinia nubilalis*), southwestern corn borer (*Diatraea grandiosella*), southern cornstalk borer (*Diatraea crambidoides*), corn earworm (*Helicoverpa zea*), fall armyworm (*Spodoptera frugiperda*), armyworm (*Pseudaletia unipunctata*), beet armyworm (*Spodoptera exigua*), black cutworm (*Agrotis ipsilon*), western bean cutworm (*Striacosta albicosta*), sugarcane borer (*Diatraea saccharalis*), and common stalk borer (*Papaipema nebris*)

c) European corn borer (*Ostrinia nubilalis*), southwestern corn borer (*Diatraea grandiosella*), southern cornstalk borer (*Diatraea crambidoides*), corn earworm (*Helicoverpa zea*), fall armyworm (*Spodoptera frugiperda*), armyworm (*Pseudaletia unipunctata*), beet armyworm (*Spodoptera exigua*), black cutworm (*Agrotis ipsilon*), western bean cutworm (*Striacosta albicosta*), sugarcane borer (*Diatraea saccharalis*), common stalk borer (*Papaipema nebris*), western corn rootworm (*Diabrotica virgifera virgifera*), northern corn rootworm (*Diabrotica barberi*), and Mexican corn rootworm (*Diabrotica virgifera zeae*)

C. Regulatory History

On November 1, 2006, EPA issued a notice in the Federal Register (71 FR 64269) announcing the filing of a pesticide tolerance petition (Pesticide Petition [PP] 6G7091) by Syngenta. The petition requested that 40 CFR Part 174 be amended by establishing a temporary exemption from the requirement of a tolerance for residues of the *Bacillus thuringiensis* Vip3Aa20 insecticidal protein when applied or used as a plant-incorporated protectant on field corn, sweet corn, and popcorn.

On November 8, 2006, EPA announced the receipt of an application for an experimental use permit (EUP) from Syngenta (71 FR 65508). The application (EPA File Symbol 67979-EUP-A) was for 536 acres of MIR162, 220 acres of *Bt*11, 199 acres of MIR604, 469 acres of *Bt*11 x MIR162, 468 acres of *Bt*11 x MIR162 x MIR604, and 1,207 acres of non plant-incorporated protectant border areas. Breeding and observation, efficacy evaluation, agronomic observation, inbred and hybrid seed production, and regulatory studies were proposed as trial protocols.

On April 4, 2007, EPA established a temporary exemption from the requirement of a tolerance for Vip3Aa20 when used as a plant-incorporated protectant in the food and feed commodities of corn (72 FR 16277; 40 CFR § 174.458). The temporary tolerance exemption was originally set to expire on March 31, 2008; however, on December 5, 2007, the EPA extended the expiration date of the temporary tolerance exemption for Vip3Aa20 until October 31, 2009 (72 FR 68525).

Subsequent to the issuance of the temporary exemption from the requirement of a tolerance for Vip3Aa20, EPA announced the issuance of the above-described EUP (EPA Registration [Reg.] Number [No.] 67979-EUP-6) in the Federal Register of June 20, 2007 (72 FR 34009). The EUP was effective from March 21, 2007 to March 31, 2008, but Syngenta later requested to extend and amend this EUP. On August 1, 2007, EPA announced receipt (72 FR 42078) of Syngenta's request to extend their EUP to October 31, 2009 and to amend it by allowing for planting of an additional 4,844 acres in 2008 (i.e., 659 acres of MIR162, 465 acres of *Bt*11, 465 acres of MIR604, 575 acres of *Bt*11 x MIR162, 575 acres of *Bt*11 x MIR604, 132 acres of MIR162 x MIR604, 575 acres of *Bt*11 x MIR162 x MIR604, and 1,398 acres of non plant-incorporated protectant border acres) and an additional 4,856 acres in 2009 (i.e., 660 acres of MIR162, 466 acres of *Bt*11, 466 acres of MIR604, 576 acres of *Bt*11 x MIR162, 576 acres of *Bt*11 x MIR604, 135 acres of MIR162 xMIR604, 576 acres of *Bt*11 x MIR162 x MIR604, and 1,401 acres of non plant-incorporated protectant border areas). The amendment to this EUP was approved on November 19, 2007.

On May 17, 2007, Syngenta submitted applications to register MIR162 maize (EPA File Symbol 67979-RU), *Bt*11 x MIR162 corn (EPA File Symbol 67979-RE), and *Bt*11 x MIR162 x MIR604 corn (EPA File Symbol 67979-RG) under Section 3 of the Federal Insecticide, Fungicide and Rodenticide Act. On July 23, 2008, the EPA announced receipt of these applications to register pesticide products containing a new active ingredient (73 FR 42799).

Concurrent with their registration applications and under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended by the Food Quality Protection Act (FQPA) of 1996, Syngenta submitted a petition to establish an exemption from tolerance for *Bacillus thuringiensis* Vip3Aa19 and Vip3Aa20 insecticidal proteins in cotton and corn when used as plant-incorporated protectants (PP 7F7217). On August 10, 2007, Syngenta submitted an amendment to their original petition to include all plants, not just cotton and corn. Although Syngenta requested simply to amend their original petition, EPA assigned the petition a new PP Number of 7F7254. Due to Syngenta's request to amend the petition, which resulted in a PP Number change, EPA considers that Syngenta administratively withdrew PP 7F7217 on August 10, 2007. On September 9, 2007, Syngenta again requested to amend their petition by asking that all variants of the *Bacillus thuringiensis* Vip3A proteins be included in the tolerance exemption. On November 2, 2007, EPA announced in the Federal Register (72 FR 62237) that Syngenta proposed to establish an exemption from the requirement of a tolerance for residues of the plant-incorporated protectant, *Bacillus thuringiensis* Vip3A proteins, when used in all crops and agricultural commodities. After review of the supporting

data, EPA determined that the permanent tolerance exemption would be limited to corn and cotton (Vip3Aa).

On August 6, 2008, the Agency established a permanent exemption from the requirement of a tolerance for residues of the *Bacillus thuringiensis* Vip3Aa proteins in or on corn and cotton (40 CFR § 174.501) when used as plant-incorporated protectants (73 FR 45620).

On November 26, 2008, a conditional registration was issued for MIR162 maize (EPA Reg. No. 67979-14).

On February 13, 2009, conditional registrations were issued for *Bt*11 x MIR162 corn (EPA Reg. No. 67979-12) and *Bt*11 x MIR162 x MIR604 corn (EPA Reg. No. 67979-13).

II. Science Assessment

Notes: The Environmental Protection Agency's science assessments often refer to Vip3Aa proteins, which include the Vip3Aa20 insecticidal protein as expressed in corn. Additionally, throughout part II of this Biopesticides Registration Action Document (BRAD), maize and corn are used interchangeably but hold the same meaning.

The classifications that are found for each data submission are assigned by Environmental Protection Agency (EPA) science reviewers and are an indication of the usefulness of the information contained in the documents for risk assessment. A rating of "ACCEPTABLE" indicates the study is scientifically sound and is useful for risk assessment. A "SUPPLEMENTAL" rating indicates the data provide some information that can be useful for risk assessment. The studies may have certain aspects determined not to be scientifically acceptable ("SUPPLEMENTAL: UPGRADABLE"). If a study is rated as "SUPPLEMENTAL: UPGRADABLE," the Environmental Protection Agency always provides an indication of what is lacking or what can be provided to change the rating to "ACCEPTABLE." If there is simply a "SUPPLEMENTAL" rating, the reviewer will often state that the study is not required by the current 40 Code of Federal Regulations (CFR) Part 158. Both "ACCEPTABLE" and "SUPPLEMENTAL" studies may be used in the risk assessment process as appropriate. An "UNACCEPTABLE" rating indicates that new data need to be submitted.

A. Product Characterization

1. MIR162 Maize (Office for Economic Cooperation and Development [OECD] Unique Identifier: SYN-IR162-4) Expressing Vip3Aa20

Vip3A is a group of vegetative insecticidal proteins (Vips) from *Bacillus thuringiensis* (*Bt*), a gram-positive bacterium commonly found in soil. Vips are produced during the vegetative stage of bacterial growth. Vip3Aa proteins are active against 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*).

The native Vip3Aa protein, Vip3Aa1, was isolated from *Bt* strain AB88 and characterized by Estruch *et al*. in 1996. Syngenta Seeds, Incorporated (hereafter referred to as Syngenta) has engineered a variant of the native gene for incorporation into corn. This engineered gene as expressed in MIR162 maize has been designated *vip3Aa20*, and it has been stably incorporated (*via* elements of vector pNOV1300) into the genome of Event MIR162 maize by *Agrobacterium*-mediated transformation. The Vip3Aa20 insecticidal protein encoded by this gene is approximately 89 kiloDaltons (kDa) molecular weight and 789 amino acids in length, differing by two amino acids from the native Vip3Aa1. The sequence differences occur at positions 129 and 284 (M129I and

K284Q, respectively). Another variant of Vip3Aa is also present as a plant-incorporated protectant (PIP) in Syngenta Event COT102 cotton (EPA Registration [Reg.] Number [No.] 67979-9) and Event Pacha corn; this variant has been assigned the designation Vip3Aa19. Vip3Aa19 differs from the native Vip3Aa1 sequence by one amino acid at position 284, while differing from Vip3Aa20 by one amino acid at position 129 (Crickmore *et al*. 2009). These substitutions are conservative and do not materially impact insecticidal activity. In fact, Vip3Aa20 shares >99.7% amino acid sequence identity with the native protein (Vip3Aa1) and Vip3Aa19.

Table 1. Comparison of Amino Acid Residues at Positions 129 and 284 in Different Variants of Vip3Aa Protein

Source of Vip3Aa Proteins	Toxin Designation	Amino Acids	Position 129*	Position 284*
Bacillus thuringiensis strain AB88	Vip3Aa1	789	M	K
COT102 Cotton	Vip3Aa19	789	M	Q
Pacha Corn	Vip3Aa19	789	M	Q
MIR162 Corn	Vip3Aa20	789	I	Q

* M = methionine, I = isoleucine, K = lysine, Q = glutamine

A *Bacillus thuringiensis* Vip3Aa20 insect control protein was produced in transgenic corn plants derived from transformation Event MIR162. A *vip3Aa20* gene was synthetically created to optimize expression in corn with activity against several major lepidopteran corn pests. Introduced *via* elements of transformation vector pNOV1300, a *vip3Aa19*-specific probe, consisting of 2,370 base pairs (bp), was incorporated between a promoter region from the *Zea mays* polyubiquitin gene (ZmUbiInt; 1,993 bp) and a terminator sequence from the 35S RNA from the cauliflower mosaic virus genome. An *Escherichia coli manA* gene encoding a phosphomannose isomerase *pmi* gene (1,176 bp) was incorporated between the same promoter region from the *Z. mays* polyubiquitin gene (1,993 bp) and a terminator sequence from the nopaline synthase gene (NOS; 253 bp) of *Agrobacterium tumefaciens*, which was used to provide a polyadenylation site. The *pmi* gene, which was introduced *via* the same pNOV1300 transformation vector, encodes the enzyme phosphomannose isomerase (PMI), which was employed as a selectable marker during the process of regenerating plant material following transformation. The PMI protein is a common enzyme involved in carbohydrate metabolism and allows for selection of transformants in cell culture, by only allowing transformed corn cells to utilize mannose as a sole carbon source, while corn cells lacking the *pmi* gene fail to grow. 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.

Data have been submitted demonstrating equivalency among the Vip3Aa1, Vip3Aa19, and Vip3Aa20 insecticidal proteins and their respective protein test substances, expressed in recombinant *E. coli* (VIP3A-0199, VIP3A-0100, VIP3A-0104, and VIP3A-0204) or maize (LPPACHA-0199, LPMIR162-0105, and IAPMIR162-0105), for use as a surrogate in toxicity experiments (see Master Record Identification [MRID] Numbers 458358-12, 468648-03, 468648-04, 468648-05, and 468648-06). Since equivalency has been established for the Vip3Aa protein variants, all previously submitted data from Vip3Aa1 and Vip3Aa19 can be bridged to Vip3Aa20.

The data submitted for product characterization for MIR162 maize are summarized in Table 2 below.

Table 2. Product Characterization Data Submitted for MIR162 Maize (Reviewed in Barsoum and Kough 2008 Unless Otherwise Noted)

Study Type/Title	Summary	MRID No.
Characteristics of *Bacillus thuringiensis* VIP3A Protein and VIP3A Cotton Plants Derived from Event COT102[a]	The *Bacillus thuringiensis* VIP3A insect control protein, as expressed in transgenic cotton seed, confers protection against the bollworm complex and other lepidopteran cotton pests. The seeds are derived from transgenic cotton event COT102, which contains the insecticidal gene *via* plasmid vector pCOT1. The product active ingredient is ≤0.0015% dry weight *Bacillus thuringiensis* VIP3A protein and the genetic material necessary for its production (pCOT1 in cotton). The product also contains ≤0.0001% dry weight marker protein and the genetic material necessary for its production (pCOT1 in cotton). VIP3A protein in transgenic cotton plants, derived from Event COT102, is produced by a synthetic *vip3A(a)* gene, which encodes a polypeptide of 789 amino acids. The VIP3A toxin is proteolytically activated to a toxin core in the lepidopteran larval midgut and forms pores in the gut membranes of sensitive species. Several formulated microbial *Bt* products containing VIP3A-like proteins and the genetic components in plasmid pCOT1, as well as the expression analysis, are described in MRID Number (No.) 457665-01. **Classification: ACCEPTABLE**	457665-01
Characterization of VIP3A Protein Produced in COT102-Derived Cotton and Comparison with VIP3A Protein Expressed in Both Maize (Corn) Derived from Event Pacha and	VIP3A protein produced in cotton plants derived from transgenic cotton event "COT102" was characterized for its biochemical and functional similarity with VIP3A expressed in recombinant *Escherichia coli* and "Pacha"-derived transgenic maize plants. Samples of purified VIP3A protein from *E. coli* and maize were dissolved in buffer for analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting. VIP3A from cotton leaves was extracted following published procedures and prepared for SDS-PAGE and western blotting. VIP3A proteins from all three sources were determined to have the	458358-12

[a] Study submitted with experimental use permit (EUP) request and reviewed in memorandum from C. Wozniak, Ph.D. to L. Cole dated March 24, 2004.

Study Type/Title	Summary	MRID No.
Recombinant *Escherichia coli*[a]	predicted molecular weight of *circa* (*ca.*) 89,000 and cross-reacted immunologically with the same anti-VIP3A antibody. Amino acid sequences corresponded identically to the predicted amino acid sequence of VIP3A and no evidence of any post-translational modification of VIP3A was observed. Peptides representing *ca.* 85% (673/789) of the complete VIP3A amino acid sequence were identified by mass spectral analysis of cotton-produced VIP3A protein. Comparisons of bioactivity of *E. coli*-expressed and cotton-expressed VIP3A protein in larvae of four lepidopteran species demonstrated comparable activities. These data indicate that VIP3A proteins from recombinant *E. coli*, Pacha-derived maize, and event COT102-derived cotton are substantially equivalent. **Classification: ACCEPTABLE**	
Molecular Characterization of Event MIR162 Maize[b]	The purpose of this study was to present molecular characterization data of the T-DNA insert and the genetic material required for its production (*via* elements of pNOV1300) in MIR162 plants. Southern blot analysis and DNA sequencing showed that the Event MIR162 maize genome contains single copies of the *vip3A* and *phosphomannose isomerase* (*pmi*) genes but no backbone sequences from the transformation plasmid, pNOV1300. Event MIR162 DNA had two single nucleotide changes in the *vip3Aa* coding sequence compared to the *vip3Aa* in pNOV1300, and was designated *vip3Aa20*. The substitution of thymine for guanine at base 387 changed the methionine at position 129 to isoleucine (M129I), but the substitution of cytosine for guanine at base 1,683 was a silent mutation. The *pmi* coding sequence in Event MIR162 was identical to that in pNOV1300. The stability of the transgenic locus was shown by statistical analysis of the Event MIR162 segregation patterns over three generations, which confirmed the expected Mendelian inheritance ratio for both the *vip3Aa20* and *pmi* genes. **Classification: SUPPLEMENTAL BUT UPGRADEABLE pending submission of an additional Southern blot containing genomic DNA from MIR162, a negative control, the plasmid control (pNOV1300) hybridized with the *pmi*-specific probe, and use of a different molecular weight marker to avoid non-specific sequence binding.** **SUPERSEDED BY MRID No. 471378-04**	468648-01
Characterization of the Vip3A Protein Expressed in Event MIR162-Derived Maize (Corn) and Comparison with Microbially Produced and Plant-	The purpose of this study was to determine if Vip3Aa20 expressed in maize plants derived from transformation Event MIR162 is substantially equivalent to Vip3Aa19 or Vip3Aa1 present in various test substances previously used in toxicity and/or test substance characterization studies. Vip3Aa proteins produced in recombinant *E. coli*, MIR162 maize, and Pacha maize were shown to be substantially equivalent based on the finding that (1) Vip3Aa20 from MIR162 maize (test material LPMIR162-0105 and	468648-02

[a] Study submitted with experimental use permit (EUP) request and reviewed in memorandum from C. Wozniak, Ph.D. to L. Cole dated March 24, 2004.

[b] Study submitted with EUP request (Event MIR162 corn) and reviewed in memorandum from A. Waggoner, through J. Kough, Ph.D., to M. Mendelsohn dated February 8, 2007.

Study Type/Title	Summary	MRID No.
Derived Vip3A Test Substances[b]	IAPMIR162-0105), Vip3Aa19 from Pacha maize (LPPACHA-0199), Vip3Aa19 from several *E. coli*-derived samples (VIP3A-0204, VIP3A-0104, VIP3A-0100), and Vip3Aa1 from *E. coli*-produced VIP3A-0199 each had the expected predicted molecular weight of ~89 kDa, and were immunoreactive with the same anti-Vip3Aa antibody on western blots, and (2) VIP3A-0204 and LPMIR162-0105 had comparable insecticidal activity against fall armyworm (137 nanograms [ng] Vip3Aa19/square centimeter [cm^2] diet surface vs. 154 ng Vip3Aa20/cm^2 diet surface). Additionally, there was no evidence of post-translational glycosylation of Vip3Aa from LPMIR162-0105 or VIP3A-0204. Therefore, the *E. coli*-produced Vip3A is considered an appropriate substitute for Vip3Aa20 expressed in MIR162 maize in toxicity and/or protein characterization studies. It was also noted that the VIP3A-0204 Vip3Aa19 protein N-terminal amino acid sequence matched the predicted sequence; however, that of plant-expressed Vip3A was not determined due to technical difficulties. **Classification: ACCEPTABLE**	
Characterization of Vip3A Protein Test Substance (VIP3A-0104) and Certificate of Analysis[b]	The purpose of this study was to characterize test substance VIP3A-0104 containing the vegetative insecticidal protein, VIP3A, encoded by the synthetic *vip3A(a)* gene. VIP3A-0104 test material (Vip3Aa19 insecticidal protein) produced from the synthetic *vip3A(a)* gene in an *E. coli* over-expression system was purified by ammonium sulfate precipitation, phenyl sepharose interaction chromatography, and diethyl aminoethyl (DEAE) anion exchange chromatography. The VIP3A-0104 samples were determined to be 63.1% pure by SDS-PAGE in conjunction with Coomassie blue staining and densitometric analysis, and were shown to contain ~2 micrograms (µg) *E. coli* endotoxin/gram (g) VIP3A-0104 by lipopolysaccharide analysis. Western blots, using goat anti-VIP3A polyclonal primary antibody and donkey anti-goat alkaline phosphatase-linked secondary antibody, revealed a dominant immunoreactive band at the predicted molecular weight of ~89,800 Daltons (Da). VIP3A-0104 had insecticidal activity against first-instar fall armyworm (FAW) larvae in insect feeding assays, with an LC_{50} of 272 ng VIP3A/cm^2 diet surface (95% confidence interval of 184–384 ng VIP3A/cm^2 diet surface) after 168 hours. **Classification: ACCEPTABLE**	468648-03
Characterization of Vip3A Protein Test Substance (VIP3A-0204) and Certificate of Analysis[b]	The purpose of this study was to characterize test substance VIP3A-0204 containing the vegetative insecticidal protein, VIP3A, encoded by the synthetic *vip3A(a)* gene. VIP3A-0204 test material (Vip3Aa19 insecticidal protein) produced from the synthetic *vip3A(a)* gene in an *E. coli* over-expression system was purified by Q Sepharose FF anion exchange chromatography. The VIP3A-0204 test material was determined to be soluble in aqueous solution at 50 milligrams (mg)/milliliter (mL), to be 89.7% pure by SDS-PAGE in conjunction with Coomassie blue staining and	468648-04

[b] Study submitted with EUP request (Event MIR162 corn) and reviewed in memorandum from A. Waggoner, through J. Kough, Ph.D., to M. Mendelsohn dated February 8, 2007.

Study Type/Title	Summary	MRID No.
	densitometric analysis, and to contain ~62 µg *E. coli* endotoxin/g VIP3A-0204 by lipopolysaccharide analysis. Western blots, using goat anti-VIP3A polyclonal primary antibody and donkey anti-goat alkaline phosphatase-linked secondary antibody, revealed a dominant immunoreactive band at the predicted molecular weight of ~89,800 Da. VIP3A-0204 had insecticidal activity against first-instar fall armyworm (FAW) larvae in insect feeding assays, with an LC_{50} of 45.1 ng VIP3A/cm^2 diet surface (95% confidence interval of 24.5–71.0 ng VIP3A/cm^2 diet surface) after 120 hours. **Classification: ACCEPTABLE**	
Re-Characterization of Vip3A Protein Test Substance (VIP3A-0204) and Certificate of Analysis[b]	The purpose of this study was to re-characterize test substance VIP3A-0204 containing the vegetative insecticidal protein, VIP3A, encoded by the synthetic *vip3A(a)* gene. VIP3A-0204 test material (Vip3Aa19 insecticidal protein) produced from the synthetic *vip3A(a)* gene in an *E. coli* over-expression was previously purified and characterized (MRID No. 468648-04). It was shown to be ~89,800 Da and 89.7% pure (SDS-PAGE with Coomassie blue staining and densitometric analysis), immunoreactive with anti-VIP3A antibody (western blots), and to have insecticidal activity against first-instar fall armyworm (FAW) larvae (LC_{50} of 45.1 ng VIP3A/cm^2 diet surface after 120 hours). In the present study, this VIP3A-0204 sample was similarly re-characterized after seven months storage lyophilized at -20°C, and shown to have retained its integrity and bioactivity. SDS-PAGE and western analysis determined a molecular weight of ~89,800 Da and a purity of 91.8%, and insecticidal activity assays with FAW larvae found an LC_{50} of 38.1 ng VIP3A/cm^2 diet surface after 120 hours. Therefore, it can be concluded that the test substance was stable when stored at -20°C over *ca.* seven months. **Classification: ACCEPTABLE**	468648-05
Characterization of VIP3A Protein Produced in Pacha-Derived Maize (Corn) and Comparison with VIP3A Protein Expressed in Recombinant *E. coli*[b]	The purpose of this study was to demonstrate the equivalency of the VIP3A protein as expressed in recombinant bacteria and transgenic maize plants derived from the Pacha VIP3A Event. Functional and biochemical parameters were evaluated and compared in order to justify the use of the microbially produced VIP3A test substance as a surrogate for maize-expressed VIP3A protein in safety evaluations. Comparisons indicated that VIP3A protein produced by Pacha-derived maize (LPPACHA-0199 sample; Vip3Aa19 insecticidal protein) and by *E. coli* (VIP3A-0199 sample; Vip3Aa1 insecticidal protein) were substantially equivalent. SDS-PAGE and western blot analysis showed that both proteins had a molecular weight (MW) of ~89,000 and were immunoreactive against the same anti-VIP3A antibody. Edman degradation was used to determine that the N-terminus of *E. coli* VIP3A was MNKN, beginning with methionine-1, and of maize VIP3A was KNNXKL, beginning with lysine-3 (X indicates that a definitive amino acid could not be assigned). The lack of two predicted amino acids at the N-terminus of maize VIP3A was likely due to proteolytic degradation *in planta* or *in vitro*. The two VIP3A proteins had a similar insecticidal activity	468648-06

[b] Study submitted with EUP request (Event MIR162 corn) and reviewed in memorandum from A. Waggoner, through J. Kough, Ph.D., to M. Mendelsohn dated February 8, 2007.

Study Type/Title	Summary	MRID No.
	profile against first-instar larvae, the *E. coli*-derived protein being slightly more active. Both were the most active against black cutworm, with estimated 96-hour LC_{50} values of 70.4 and 88.5 ng VIP3A/cm^2 diet surface for the *E. coli* VIP3A and the maize VIP3A, respectively. Both were slightly less active against corn earworm and fall armyworm, and as expected, were inactive against the European corn borer and diamondback moth. Mass spectral (MS) analysis of VIP3A proteolytic digests confirmed the predicted complete sequence of ~95% of the *E. coli* and ~93% of the maize VIP3A protein. Neither MS analysis nor an independent glycosylation analysis (DIG Glycan) showed any evidence of post-translational glycosylation of either the microbially derived or maize-derived VIP3A. **Classification: SUPPLEMENTAL BUT UPGRADEABLE pending submission of better reproductions of the SDS-PAGE and western blots of the *E. coli*- and maize-derived VIP3A test proteins as well as clarifications to data discrepancies noted in the insect bioassay.**	
Analytical Method for the Detection of Vip3Aa20 Protein in Maize Tissues from Event MIR162[b]	An enzyme-linked immunosorbent assay (ELISA)(Tijssen 1985) procedure was used to determine Vip3Aa20 levels in tissues of Event MIR162 maize and the Vip3Aa20 insecticidal protein tissue extraction efficiency. The ELISA method used 96-well plates, purified rabbit anti-VIP3A polyclonal primary antibody, donkey anti-rabbit alkaline phosphatase conjugated secondary antibody, and phosphatase substrate. Each plate included the standard test substance (MIR162-VIP3A-0106 or VIP3A-0104) that was used to generate a standard curve, but these data were not shown. The limit of quantitation (LOQ) and limit of detection (LOD) for Vip3Aa20 ranged from, respectively, 0.04–0.25 and 0.003–0.032 µg Vip3Aa20/g fresh weight, to 0.21–0.35 and 0.029–0.045 µg Vip3Aa20/g dry weight. The average extraction efficiency of Vip3Aa20 was 82.7% in leaves, 81.0% in roots, 79.5% in pith, 88.3% in silk, 79.7% in kernels, >98% in pollen, and 78.9% in whole plants at maturity. **Classification: UNACCEPTABLE for residue analytical method. A new study should be submitted (concurrently with the Section 3 registration of Event MIR162) and specifically conducted on the MIR162 transgenic grain (single seed and composite) in order to be verified as a suitable analytical method. This experiment should also be validated by an independent third party laboratory according to Office of Prevention, Pesticides, and Toxic Substances (OPPTS) Guideline 860.1340(c)(6) and Pesticide Registration (PR) Notice 96-1 with Good Laboratory Practice (GLP) standards compliance. The report should also include the following items: (1) Qualitative data to represent positive vs. negative transgenic specific event results with percent accuracy; (2) Utilization of a negative control (non-transgenic convention corn line) and positive control (confirmed transgenic corn line); (3) Testing of cross-reactivity**	468648-07

[b] Study submitted with EUP request (Event MIR162 corn) and reviewed in memorandum from A. Waggoner, through J. Kough, Ph.D., to M. Mendelsohn dated February 8, 2007.

Study Type/Title	Summary	MRID No.
	against other transgenic events and other proteins; and (4) The intra- and inter-assay coefficient of variation should be reported. **Once the recommended report has been submitted and found acceptable, EPA's Analytical Method Laboratory in Fort Meade, Maryland will have to independently validate Syngenta's ELISA protocol for accuracy, precision, and sensitivity.** **SUPPLEMENTAL with regards to establishing field protein expression levels of MIR162 corn tissues and plants. It does provide useful information for tissue expression levels to determine exposure for non-target organisms, for insect resistance management (IRM) dose levels, and dietary exposure estimates. However, it does not include quantification of Vip3Aa20 insecticidal protein levels expressed in various plant tissues and the whole corn plant. A full report determining the protein concentrations of Vip3Aa20 and PMI at different stages of plant development should be submitted (including: the mean, range, and standard deviations) and reported on a dry weight basis (µg protein/g tissue) with GLP standards compliance. This data requirement can be addressed in the Section 3 registration of Vip3Aa20. The study should also include the following items: (1) Standard curve data for the ELISA; (2) The calculation method for determining the dry weight conversion factor from the fresh weight tissue samples; and (3) Identification of the specific seed line and lot utilized as the test material with number of field sites and replicates.** **SUPERSEDED BY MRID NUMBERS 471378-05, 471378-06, and 471378-07**	
The Mode of Action of the *Bacillus thuringiensis* Vegetative Insecticidal Protein Vip3A Differs from that of Cry1Ab δ-Endotoxin[b]	This publication (Lee *et al*. 2003), which examined the differences in the mechanism of insecticidal activity of Cry1Ab and Vip3A, was submitted by the registrant to provide additional product characterization data, specifically Vip3A's mode of action. The submitted publication examined differences in the mechanism of insecticidal activity of Cry1Ab and Vip3A proteins. Ligand blotting showed that activated Cry1Ab and Vip3A-G (Vip3A proteolytically cleaved with lepidopteran gut juice) bound different receptor molecules in the midgut of tobacco hornworm (*Manduca sexta*, Linnaeus) and that Vip3A-G did not bind Cry1A receptors. Voltage clamping assays showed that Vip3A-G formed distinct pores in dissected midgut from *M. sexta* but not in the monarch butterfly (*Danaus plexippus*, Linnaeus). Cry1Ab and Vip3A both formed voltage-independent and cation-selective stable ion channels in planar lipid bilayers, but their primary conductance state and cation specificity differed.	468808-01

[b] Study submitted with EUP request (Event MIR162 corn) and reviewed in memorandum from A. Waggoner, through J. Kough, Ph.D., to M. Mendelsohn dated February 8, 2007.

Study Type/Title	Summary	MRID No.
	Classification: ACCEPTABLE	
Characterization of Microbially Produced Vip3A in Event MIR162-Derived Maize	This study compared the similarity of Vip3Aa20 insecticidal protein expressed in corn event MIR162 with that expressed in recombinant *E. coli*. The Vip3Aa20 insecticidal protein derived from corn event MIR162 and recombinant *E. coli* had the same approximate molecular weight, which was *ca.* 89 kDa. The Vip3Aa20 insecticidal proteins from both sources were immunologically cross-reactive with the same anti-Vip3A antibody. Both proteins produced comparable toxicities toward fall armyworm (*S. frugiperda*, FAW) larvae, based on LC_{50} values. There was no evidence of post-translational glycosylation of the microbially derived Vip3Aa20 insecticidal protein, consistent with Vip3Aa20 from MIR162 maize. Peptide mass mapping analysis provided additional strong evidence of the identity and equivalence of Vip3Aa20 expressed in event MIR162 maize and in bacterially derived Vip3Aa20. The microbially produced test substance, MIR162VIP3A-0106, is a suitable surrogate for Vip3Aa20 expressed in MIR162 maize. **Classification: ACCEPTABLE**	471378-01
Characterization of Phosphomannose Isomerase (PMI) Produced in Event MIR162 Maize and Comparison to PMI as Contained in Substance PMI-0198	The PMI enzyme produced in transgenic Event MIR162 maize was compared to the PMI enzyme present in test substance PMI-0198 produced *via* a recombinant *Escherichia coli* over-expression system. The PMI proteins from the two sources were demonstrated to have the predicted molecular weights of *ca.* 42.8 kDa for the plant-expressed PMI and *ca.* 44.4 kDa for the microbially expressed PMI (contains 16 additional, non-functional, amino acids at the N-terminus of the protein). Both PMI proteins cross-reacted with the same anti-PMI antibodies. PMI in the microbial test substance, PMI-0198, was found to have a specific enzymatic activity of *ca.* 33.2 U/mg PMI and the plant-expressed enzyme showed a specific activity of *ca.* 55.5 U/mg PMI. Based on these results, it was concluded that the PMI proteins in test substance PMI-0198 and Event MIR162-derived maize are biochemically and functionally equivalent, and that PMI in test substance PMI-0198 is a suitable surrogate for PMI protein produced in Event MIR162 maize. **Classification: ACCEPTABLE**	471378-02
Molecular Characterization of the Transgenic DNA in Event MIR162 Maize	This study characterized the T-DNA insert in Event MIR162 maize. This recombinant maize line expresses the modified *vip3Aa19* gene (*vip3Aa20*), which encodes a vegetative insecticidal protein that is highly toxic to certain lepidopteran insect pests. Additionally, these plants also contain the *pmi* gene that expresses phosphomannose isomerase, a selectable marker trait that is inert with regard to pesticidal properties. The T-DNA insert, introduced *via* the pNOV1300 plasmid in MIR162, was analyzed by Southern blots to determine the number of insertions, copy number of functional elements, presence or absence of backbone elements, and stability of the inserted DNA during breeding. DNA sequence analysis was used to assess the intactness of the insert, the continuity of the functional elements within the insert, and the presence of any rearrangements, deletions and/or	471378-04

Study Type/Title	Summary	MRID No.
	base pair changes within the insert, whether or not the insert occurred in a known functional maize gene, and whether novel, open reading frames (ORFs) were generated at the junctions of the T-DNA within the maize genome. The study found that the MIR162 maize genome contains single copies of the *vip3Aa20* and *pmi* genes, two copies of the polyubiquitin (ZmUbiInt) promoter in addition to an endogenous polyubiquitin promoter, a single copy of the NOS terminator, and no backbone sequences from pNOV1300, and the T-DNA insert is stable over several generations. Sequence analysis of the entire T-DNA insert in MIR162 maize confirmed the intactness of the insert and the continuity of the functional elements. Sequence analysis revealed two single nucleotide changes in the *vip3Aa* coding sequence contained in the MIR162 T-DNA insert, as compared with the sequence present in the transformation vector pNOV1300. **Classification: ACCEPTABLE**	
Quantification of Vip3Aa20 and Phosphomannose Isomerase (PMI) in Tissues of Maize Derived from Transformation Event MIR162	The purpose of this study was to determine the concentrations of the Vip3Aa20 and PMI proteins expressed in MIR162 maize hybrids and near-isogenic, non-transgenic controls grown in field trials. Concentrations of Vip3Aa20 and PMI were determined by ELISA for various plant tissues and developmental stages in two MIR162 hybrids, one grown in Bloomington, Illinois and the other in York, Nebraska. Quantities of Vip3Aa20 and PMI present on a per-acre and per-hectare basis were estimated for four stages of plant development for these same maize hybrids. For all assessments, near-isogenic, non-transgenic control plant tissues were concurrently collected and analyzed in a similar manner to test for lack of Vip3Aa20 and PMI expression or interference from background substances. For both MIR162 maize hybrids, both Vip3Aa20 and PMI concentrations were quantifiable in all tissues for at least one growth stage. Both MIR162 maize hybrids (A and B) expressed similar concentrations of the Vip3Aa20 insecticidal protein. PMI concentrations in the two MIR162 maize hybrids (A and B) were also comparable. Estimates of mean Vip3Aa20 quantities in the transgenic plants on a per-acre (and per-hectare) basis were highest at seed maturity. Vip3Aa20 and PMI concentrations in the near-isogenic, non-transgenic negative control samples were either below the limit of detection (<LOD) or quantitation (<LOQ). **Classification: ACCEPTABLE**	471378-05
Validation of the Analytical Method for Qualitative Detection of Vip3Aa20 Protein in Maize Seed	The purpose of this study was to measure the inter- and intra-assay precision, limit of detection, accuracy, and specificity of the method described in Standard Operating Procedure (SOP) 2.91, "Extraction and Qualitative Detection of Vip3Aa20 Protein from MIR162 Maize Seed." The analyses were done using an enzyme-linked immunosorbent assay kit (SeedChek Vip3A ELISA) from Strategic Diagnostics, Incorporated. The LOD for the assay was 0.102 (optical density at 650 nanometers). Inter-assay precision ranged from 16.4% coefficient of variation (CV) to 19.0% CV and intra-assay precision ranged from 8.8% CV to 21.0% CV for the fortification levels tested (0, 1:400, and 1:800). The accuracy of the assay, evaluated by testing single seeds of MIR162 and non-transgenic maize, was determined to be 100%. No cross-reactivity was observed with other	471378-06

Study Type/Title	Summary	MRID No.
	commercially available transgenic maize hybrids. The assay was capable of detecting adventitious presence at a level of one MIR162 seed in 800 total seeds (on a weight-to-weight basis). The results of the study demonstrated the validity of SOP 2.91 for the qualitative determination of Vip3Aa20 insecticidal protein contained in maize seed. **Classification: SUPPLEMENTAL based on the following deficiencies: (1) The results of the analytical method should be reported as gram/gram and not as an optical density value and (2) dilutions from corn samples, before grinding, should be tested instead of flour samples.**	
Independent Laboratory Validation of "Extraction and Qualitative Detection of Vip3Aa20 Protein from MIR162 Maize Seed"	The purpose of this study was to validate Syngenta's SOP 2.91.1, "Extraction and Qualitative Detection of Vip3Aa20 Protein from MIR162 Maize Seed." The validation was carried out using a prototype enzyme-linked immunosorbent assay kit (SeedChek Vip3A ELISA) from Strategic Diagnostics, Incorporated. The sponsor furnished ground, non-transgenic maize flour and MIR162 transgenic maize flour. The two types of flour were mixed at ratios of 1:400 and 1:800 (by mass) transgenic to non-transgenic flour. The mixtures were extracted along with an unfortified, non-transgenic control according to SOP 2.91.1. The endpoint of the ELISA analysis was light absorption at 650 nanometers (optical density$_{650\,nm}$), indicating the presence of the Vip3A protein. The 1:400 samples produced the most intense optical densities (ODs), which exceeded 1.0 with all values ranging between 1.0928 and 1.3953. The 1:800 sample ODs were less strongly colored, ranging between 0.7003 and 0.811. All unfortified, non-transgenic controls yielded values $\leq$0.0513, which were below the limit of detection (limit of detection = 0.102). The results demonstrated that SOP 2.91.1 is a satisfactory method for the qualitative determination of non-transgenic maize flour and mixtures of non-transgenic and MIR162 transgenic maize flour containing Vip3Aa20 insecticidal protein. **Classification: ACCEPTABLE**	471378-07

2. *Bt*11 x MIR162 Corn (OECD Unique Identifier: SYN-BTØ11-1 x SYN-IR162-4) Expressing Cry1Ab and Vip3Aa20 and *Bt*11 x MIR162 x MIR604 Corn (OECD Unique Identifier: SYN-BTØ11-1 x SYN-IR162-4 x SYN-IR6Ø4-5) Expressing Cry1Ab, Vip3Aa20, and mCry3A

The *Bt*11 x MIR162 hybrid cross combines Events *Bt*11 and MIR162 by conventional breeding, while the *Bt*11 x MIR162 x MIR604 hybrid cross combines Events *Bt*11, MIR162, and MIR604 by conventional breeding. These individual events are briefly summarized in the following paragraphs, except for MIR162, which was previously described in section II(A)(1) of this chapter.

In 1996, EPA granted a registration for the *Bacillus thuringiensis* subspecies *kurstaki* strain HD-1 Cry1Ab protein and the genetic material necessary for its production in Event MON 810 corn (EPA Reg. No. 524-489). Syngenta bridged data from MON 810 and provided additional product characterization data to register Cry1Ab expressed in Event *Bt*11 corn (EPA Reg. No. 67979-1 for

field corn; EPA Reg. No. 65268-1 for sweet corn). The phosphinothricin acetyltransferase (PAT) protein is also expressed with Cry1Ab in *Bt*11 corn as a PIP inert ingredient. The product characterization data supporting the registration of *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production (*via* elements of plasmid vector pZO1502) in Event *Bt*11 corn, which includes the submitted study titles, conclusions, and their MRID Numbers, are found in the 2001 *Bt* Crops Reassessment (U.S. EPA 2001).

Syngenta's Event MIR604 corn plants were a result of a corn plant transformation with the synthetic modified *cry3A* gene, which provides resistance to western corn rootworm and northern corn rootworm. In 2006, the Agency issued a Section 3 registration (EPA Reg. No. 67979-5) for *Bacillus thuringiensis* mCry3A protein and the genetic material necessary for its production (*via* elements of plasmid vector pZM26) in Event MIR604 corn. The product characterization data supporting the registration of *Bacillus thuringiensis* mCry3A protein and the genetic material necessary for its production (*via* elements of plasmid vector pZM26) in Event MIR604 corn, which includes the submitted study titles, conclusions, and their MRID Numbers, are found in the mCry3A BRAD (U.S. EPA 2007).

The data submitted for *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn, which includes confirmation of molecular identity and protein expression levels for both stacked and/or pyramided varieties as well as a characterization report for the microbially produced TRYCRY1AB-0105 test substance, are summarized in Table 3 below.

Table 3. Product Characterization Data Submitted for *Bt*11 x MIR162 Corn and *Bt*11 x MIR162 x MIR604 Corn (Reviewed in Waggoner and Kough 2008 Unless Otherwise Noted)

Study Type/Title	Summary	MRID No.
Comparative Southern Analysis of *Bt*11 x MIR162 x MIR604 Maize Hybrid with the Individual Events *Bt*11 Maize, MIR162 Maize, and MIR604 Maize	The purpose of this study was to use Southern blot analysis to confirm the presence of the *cry1Ab* and *pat* genes from the parental Event *Bt*11, *vip3Aa20* and *pmi* genes from parental Event MIR162, and *mcry3A* and *pmi* genes from parental Event MIR604 in the hybrid, *Bt*11 x MIR162 x MIR604, in a predictable manner. For the *cry1Ab*-specific probe, *Nde*I, *Sph*I, and *Bgl*II + *Eco*RI digests of *Bt*11 DNA resulted in a single hybridization band of >4.4 kilobase (kb), >4.5 kb, and ~4.7 kb, respectively, in Event *Bt*11 and the *Bt*11 x MIR162 x MIR604 hybrid, thus, indicating the presence of the *cry1Ab* gene. Likewise, for the *pat*-specific probe, the *Nde*I, *Sph*I, and *Bgl*II + *Eco*RI digests of *Bt*11 DNA resulted in a single hybridization band of >1.7 kb, >4.5 kb, and ~4.7 kb, respectively, in Event *Bt*11 and the *Bt*11 x MIR162 x MIR604 hybrid, thus, indicating the presence of the *pat* gene. The MIR162 DNA and MIR604 DNA digested with each restriction enzyme were negative because these events do not contain the *cry1Ab* or *pat* genes.	471372-01

Study Type/Title	Summary	MRID No.
	For the *vip3A19*-specific probe[c], the *Kpn*I, *Eco*RV, and *Bam*HI digests of MIR162 DNA resulted in a single hybridization band of >4.7 kb, >6.9 kb, and ~4.6 kb, respectively, in Event MIR162 and the *Bt*11 xMIR162 x MIR604 hybrid, thus, indicating the presence of the *vip3Aa20* gene. No banding was present in either the *Bt*11 DNA or MIR604 DNA because neither event contains the *vip3Aa20* gene. For the *pmi*-specific probe, the *Kpn*I, *Bam*HI, and *Hind*III + *Xma*I digests of MIR162 DNA resulted in a single hybridization band of >3.6 kb, >1.6 kb, and ~8.1 kb, respectively, in Event MIR162, Event MIR604, and the *Bt*11 x MIR162 x MIR604 hybrid, thus, indicating the presence of the *pmi* gene. The *Bt*11 DNA digested with each restriction enzyme was negative because this event does not contain the *pmi* gene. For the *mcry3A*-specific probe, the *Kpn*I, *Eco*RV, and *Asc*I + *Xma*I digests of MIR604 DNA resulted in a single hybridization band of >4.8 kb, >7.0 kb, and ~8.2 kb, respectively, in both Event MIR604 and the stacked *Bt*11 x MIR162 x MIR604 hybrid, indicating the presence of the *mcry3A* gene. The *Bt*11 DNA digested with each restriction enzyme was negative because this event does not contain the *mcry3A* gene. Therefore, the predicted DNA hybridization patterns were retained and stability of the transgenic locus from parent to progeny was demonstrated. **Classification: ACCEPTABLE**	
Comparison of Transgenic Protein Expression in Event *Bt*11, Event MIR162, Event MIR604, and Stacked *Bt*11 x MIR162 x MIR604 Maize (Corn) Hybrids	The purpose of this study was to compare expression of each transgenic protein (Cry1Ab, PAT, Vip3Aa20, PMI, and mCry3A) in a *Bt*11 x MIR162 x MIR604 maize (field corn) hybrid with expression in corresponding near-isogenic hybrids derived from the individual transformation events. Four hybrid plants per individual maize event (*Bt*11, MIR162, and MIR604) and from a stacked hybrid of all three events (*Bt*11 x MIR162 x MIR604) were collected at different developmental stages from each of five replicate-planted blocks of maize. Plant tissue extracts from leaves, roots, pollen, and whole-plant samples at the anthesis stage and kernel samples at the physiological maturity stage were analyzed for each of the transgenic proteins from the appropriate hybrids *via* ELISA. The average concentrations of Cry1Ab insecticidal protein in the *Bt*11 hybrid were measured in comparison to the stacked *Bt*11 x MIR162 x MIR604 hybrid for each plant tissue. Tissues analyzed for Cry1Ab insecticidal protein included: leaves (92.7 to 88.4 µg/g dry weight [dw]), roots (11.5 to 11.3 µg/g dw), pollen (0.0764 to 0.0801 µg/g dw), kernels	471372-02

[c] A *vip3Aa19* probe was used for the *vip3Aa20* analysis. The nucleotide sequences of *vip3Aa19* and *vip3Aa20* differ by two nucleotides and are 99.9% identical; this should not affect the ability of the *vip3Aa19*-specific probe to hybridize to the *vip3Aa20* sequence present in MIR162 maize or its associated stacks and/or pyramids.

Study Type/Title	Summary	MRID No.
	(1.78 to 1.57 µg/g dw), and the whole plant (15.9 to 15.2 µg/g dw). The average concentrations of PAT protein in the *Bt*11 hybrid were measured in comparison to the stacked *Bt*11 x MIR162 x MIR604 hybrid for each plant tissue. Tissues analyzed for PAT protein included: leaves (0.596 to 0.603 µg/g dw), roots (0.905 to 0.739 µg/g dw), pollen (below LOD), kernels (below LOD), and the whole plant (0.912 to 0.873 µg/g dw). The average concentrations of Vip3Aa20 insecticidal protein in the MIR162 hybrid were measured in comparison to the stacked *Bt*11 x MIR162 x MIR604 hybrid for each plant tissue. Tissues analyzed for Vip3Aa20 insecticidal protein included: leaves (165.6 to 159.7 µg/g dw), roots (52.1 to 53.1 µg/g dw), pollen (97.2 to 85.4 µg/g dw), kernels (123.8 to 140.1 µg/g dw), and the whole plant (73.0 to 72.6 µg/g dw). The average concentrations of PMI protein in the MIR162 hybrid were measured in comparison to the stacked *Bt*11 x MIR162 x MIR604 hybrid of each plant tissue. Tissues analyzed for PMI protein included: leaves (7.72 to 16.3 µg/g dw), roots (2.58 to 5.37 µg/g dw), pollen (5.07 to 48.1 µg/g dw), kernels (2.48 to 5.18 µg/g dw), and the whole plant (3.87 to 8.54 µg/g dw). The average concentrations of mCry3A insecticidal protein in the MIR604 hybrid were measured in comparison to the stacked *Bt*11 x MIR162 x MIR604 hybrid for each plant tissue. Tissues analyzed for mCry3A insecticidal protein included: leaves (35.8 to 34.0 µg/g dw), roots (22.6 to 25.4 µg/g dw), pollen (below LOD), kernels (0.717 to 0.620 µg/g dw), and the whole plant (18.1 to 16.2 µg/g dw). The average concentrations of PMI protein in the MIR604 hybrid were measured in comparison to the stacked *Bt*11 x MIR162 x MIR604 hybrid of each plant tissue. Tissues analyzed for PMI protein included: leaves (5.13 to 10.0 µg/g dw), roots (2.41 to 4.08 µg/g dw), pollen (43.3 to 50.4 µg/g dw), kernels (2.33 to 4.74 µg/g dw), and the whole plant (4.37 to 7.20 µg/g dw). Overall, concentrations of Cry1Ab, Vip3Aa20, mCry3A, and PAT proteins were found comparable and all control tissues were negative for the expression of Cry1Ab, Vip3Aa20, and PAT proteins. Mean total PMI (PMI + MIR604 PMI) concentrations were consistently higher, as expected, in the tissues of the *Bt*11 x MIR162 x MIR604 hybrid as compared to PMI concentrations in the MIR162 hybrid or MIR604 PMI concentrations in the MIR604 hybrid. This reflected the inheritance of both PMI and MIR604 PMI in the *Bt*11 x MIR162 x MIR604 hybrid. Generally, the mean total PMI concentrations in the stacked hybrid was approximately double that in the individual MIR162 and MIR604 parental events. This effect is most likely due to the two sources of the *pmi* gene from the MIR162 and MIR604 parental events. Therefore, transgenic protein expression in the *Bt*11 x MIR162 x MIR604 hybrid is not substantially different from that of the hybrids derived from the individual *Bt*11, MIR162, and MIR604 transformation events. **Classification: ACCEPTABLE**	

Study Type/Title	Summary	MRID No.
Characterization of Trypsinized Cry1Ab Test Substance TRYCRY1AB-0105	The purpose of this study was to characterize test substance TRYCRY1AB-0105, containing the *ca.* 66 kDa truncated form of the full-length (*ca.* 130 kDa) Cry1Ab insecticidal protein. The identity of test substance TRYCRY1AB-0105 was evaluated using SDS-PAGE, western blot analysis, mass analysis, peptide mapping, N-terminal sequence analysis, an insecticidal bioassay, and analysis of its lipopolysaccharide (*E. coli* endotoxin) content. The test substance purity was calculated as 127 µg Cry1Ab/mL TRYCRY1AB-0105 or 74.1% Cry1Ab protein/total protein. Protein levels were determined by measuring sample absorption at 280 nm (A_{280} method) in conjunction with densitometry data after electrophoretic separation. Western blot analysis of the test substance showed a dominant immunoreactive band corresponding to the predicted molecular weight of Cry1Ab of *ca.* 66 kDa. Total mass analysis of the Cry1Ab in test substance TRYCRY1AB-0105 found two predominant Cry1Ab species, with molecular weights of 66.3 and 65.8 kDa. In addition, two putative Cry1Ab breakdown fragments corresponding to *ca.* 39 kDa and 27 kDa were present. The 65.8 and 66.3 kDa Cry1Ab protein species both contain the biologically active portion of the insecticidal Cry1Ab protein and, therefore, were considered throughout the study as active Cry1Ab protein and were included in the purity estimate of Cry1Ab protein in test substance TRYCRY1AB-0105. N-terminal sequencing of Cry1Ab in test substance TRYCRY1AB-0105 confirmed that the first 10 amino acids of the protein corresponded to the predicted N-terminal sequence of Cry1Ab. Peptide mapping of the Cry1Ab in test substance TRYCRY1AB-0105, representing 26% coverage of the Cry1Ab protein, gave peptide spectra identical to that of the known sequence of trypsinized Cry1Ab. TRYCRY1AB-0105 was also evaluated for lipopolysaccharide concentration and found to contain 4.8 ng *E. coli* endotoxin/mL test substance TRYCRY1AB-0105. Lastly, the Cry1Ab bioactivity against the European corn borer was confirmed. The 96-hour LC_{50} was 6.2 ng Cry1Ab/cm^2 diet surface (95% confidence interval: 3.8–8.9 ng/ cm^2 diet surface) against first-instar larvae of the European corn borer. **Classification: ACCEPTABLE when combined with the summary of data presented in MRID No. 476049-01.**	471372-11
Response to Data Deficiencies Noted for the *Bt*11 x MIR162 and *Bt*11 x MIR162 x MIR604 Applications for Registration (EPA	The registrant submitted the mass spectra and a clearer reproduction of the Western blot gel as visual confirmation for establishing the molecular weight of the test substance TRYCRY1AB-0105, containing the *ca*. 66 kDa truncated form of the full-length (*ca*. 130 kDa) Cry1Ab insecticidal protein. Total mass analysis of the Cry1Ab in test substance TRYCRY1AB-0105 found two predominant comigrating Cry1Ab species with molecular weights	476049-01

Study Type/Title	Summary	MRID No.
Reg. No. 67979-RE and 67979-RG)[d]	of 66.3 and 65.8 kDa. Other supporting data include: insect bioassays demonstrating similar bioactivity of the test substance against European corn borer (ECB); N-terminal amino acid sequencing analysis of TRYCRY1AB-0105 in comparison to Cry1Ab insecticidal protein expressed in Event *Bt*11; and peptide mass analysis via quadruple time-of-flight (Q-TOF) comparing the masses of individual peptides resulting from proteolytic digestion of a test sample to the masses of known peptides in a database. In addition, field trial results comparing the efficacy of the single plant-incorporated protectant events (*Bt*11, MIR162, and MIR604) to the combination PIP products, *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn, showed no enhanced toxicity among various target pest species. Collectively, these data demonstrated the equivalence between the plant- and microbial-produced Cry1Ab proteins in support of utilizing the TRYCRY1AB-0105 test substance as a suitable surrogate for the non-target organism toxicity studies submitted in support for the registration of the stacked and/or pyramided *Bt*11 x MIR162 and *Bt*11 x MIR162 x MIR604 corn PIP products. **Classification: ACCEPTABLE**	
Comparative Southern Analysis of *Bt*11 x MIR162 Hybrid with the Individual Events, *Bt*11 Maize and MIR162 Maize	The purpose of this study was to use Southern blot analysis to confirm the presence of the *cry1Ab* and *pat* genes from the parental Event *Bt*11 and *vip3Aa20* and *pmi* genes from the parental Event MIR162 in *Bt*11 x MIR162 corn in a predictable manner. For the *cry1Ab*-specific probe, the *Nde*I, *Sph*I, and *Bgl*II + *Eco*RI digests of *Bt*11 DNA resulted in a single hybridization band of >4.4 kb, >4.5 kb, and ~4.7 kb, respectively, in both Event *Bt*11 and *Bt*11 x MIR162 corn, indicating the presence of the *cry1Ab* gene. Likewise, for the *pat*-specific probe, *Nde*I, *Sph*I, and *Bgl*II + *Eco*RI digests of *Bt*11 DNA resulted in a single hybridization band of >1.7 kb, >4.5 kb, and ~4.7 kb, respectively, in both Event *Bt*11 and *Bt*11 x MIR162 corn, indicating the presence of the *pat* gene. The MIR162 DNA digested with each restriction enzyme was negative because this event does not contain the *cry1Ab* or *pat* genes. For the *vip3Aa19*-specific probe[e], the *Kpn*I, *Eco*RV, and *Nco*I digests of MIR162 DNA resulted in a single hybridization band of >4.7 kb, >6.9 kb, and ~4.6 kb, respectively, in both Event MIR162 and *Bt*11 x MIR162 corn, indicating the presence of the *vip3Aa20* gene. Likewise, for the *pmi*-specific probe, *Kpn*I, *Bam*HI, and *Hind*III + *Xma*I digests of MIR162 DNA resulted in a single hybridization band of >3.6 kb, >1.6 kb, and ~8.1 kb, respectively, in both Event MIR162 and *Bt*11 x MIR162 corn, indicating the presence of	471374-01

[d] Study submitted with Section 3 request (*Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn) and reviewed in memorandum from A. Waggoner, through J. Kough, Ph.D., to J. Kausch dated February 11, 2009.

[e] A *vip3Aa19* probe was used for the *vip3Aa20* analysis. The nucleotide sequences of *vip3Aa19* and *vip3Aa20* differ by two nucleotides and are 99.9% identical; this should not affect the ability of the *vip3Aa19*-specific probe to hybridize to the *vip3Aa20* sequence present in MIR162 maize or its associated stacks and/or pyramids.

Study Type/Title	Summary	MRID No.
	the *pmi* gene. The *Bt*11 DNA digested with each restriction enzyme was negative because this event does not contain the *vip3Aa20* and *pmi* genes. Therefore, the predicted DNA hybridization patterns were retained and stability of the transgenic locus from parent to progeny was demonstrated. **Classification: ACCEPTABLE**	
Comparison of Transgenic Protein Expression in Event *Bt*11, Event MIR162 and Stacked *Bt*11 x MIR162 Maize (Corn) Hybrids	The purpose of this study was to compare expression of the four transgenic proteins (Cry1Ab, Vip3Aa20, PAT, and PMI) in a *Bt*11 x MIR162 corn hybrid with expression in corresponding near-isogenic hybrids derived from the individual transformation events. Four plants per each parental-event hybrid (*Bt*11 and MIR162) and the stacked hybrid (*Bt*11 x MIR162 corn) were collected at different developmental stages from each of five replicate-planted blocks of maize. Plant tissue extracts from leaves, roots, pollen, and whole-plant samples at the anthesis stage and kernel samples at the physiological maturity stage were analyzed for each of the transgenic proteins from the appropriate hybrids via enzyme-linked immunosorbent assay. The average concentrations of Cry1Ab insecticidal protein in the *Bt*11 hybrid were measured in comparison to the stacked *Bt*11 x MIR162 hybrid for each plant tissue. Tissues analyzed for Cry1Ab insecticidal protein included: leaves (141.7 to 154.2 µg/g dw), roots (12.8 to 11.9 µg/g dw), pollen (0.636 to 0.858 µg/g dw), kernels (6.94 to 6.79 µg/g dw), and the whole plant (19.6 to 17.8 µg/g dw). The average concentrations of PAT protein in the *Bt*11 hybrid were measured in comparison to the stacked *Bt*11 x MIR162 hybrid for each plant tissue. Tissues analyzed for PAT protein included: leaves (0.657 to 0.629 µg/g dw), roots (0.580 to 0.403 µg/g dw), pollen (below LOD), kernels (below LOD), and the whole plant (0.872 to 0.751 µg/g dw). The average concentrations of Vip3Aa20 insecticidal protein in the MIR162 hybrid were measured in comparison to the stacked *Bt*11 x MIR162 hybrid for each plant tissue. Tissues analyzed for Vip3Aa20 insecticidal protein included: leaves (185.0 to 191.6 µg/g dw), roots (32.0 to 28.4 µg/g dw), pollen (107.6 to 157.0 µg/g dw), kernels (83.8 µg/g dw for both), and the whole plant (80.4 to 79.0 µg/g dw). The average concentrations of PMI protein in the MIR162 hybrid were measured in comparison to the stacked *Bt*11 x MIR162 hybrid for each plant tissue. Tissues analyzed for PMI protein included: leaves (6.74 to 7.44 µg/g dw), roots (2.03 to 2.15 µg/g dw), pollen (4.62 to 4.79 µg/g dw), kernels (1.84 to 1.77 µg/g dw), and the whole plant (3.94 µg/g dw for both). Overall, concentrations of Cry1Ab, Vip3Aa20, PAT, and PMI protein were found comparable and all control tissues were negative for the expression of Cry1Ab, Vip3Aa20, PAT, and PMI proteins. Therefore, transgenic protein	471374-02

Study Type/Title	Summary	MRID No.
	expression in the *Bt*11 x MIR162 hybrid are not substantially different from that of the hybrids derived from the individual *Bt*11 and MIR162 transformation events. **Classification: ACCEPTABLE**	

B. Human Health Assessment

1. Human Health Assessment of Vip3Aa Proteins

i. Mammalian Toxicity and Allergenicity Assessment

Consistent with Section 408(b)(2)(D) of the Federal Food, Drug, and Cosmetic Act (FFDCA), EPA has reviewed the available scientific data and other relevant information in support of this action and considered its validity, completeness and reliability and the relationship of this information to human risk. EPA has also considered available information concerning the variability of the sensitivities of major identifiable subgroups of consumers, including infants and children.

Syngenta has submitted acute oral toxicity data demonstrating the lack of mammalian toxicity at high levels of exposure to Vip3Aa proteins. These data demonstrate the safety of Vip3Aa proteins at a level well above maximum possible exposure levels that are reasonably anticipated in the crops. Basing this conclusion on acute oral toxicity data without requiring further toxicity testing and residue data is similar to the Agency position regarding toxicity testing and the requirement of residue data for the microbial *Bacillus thuringiensis* products from which this plant-incorporated protectant was derived (see 40 CFR § 158.2140). For microbial products, further toxicity testing (Tiers II and III) and residue data are triggered by significant adverse acute effects in studies such as the mouse oral toxicity study, to verify the observed adverse effects and clarify the source of these effects.

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 LD_{50} 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 a new 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).

When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Sjoblad *et al*. 1992). Therefore, since no acute effects were shown to be caused by the Vip3Aa19 and Vip3Aa20 insecticidal proteins, even at relatively high dose levels, they are not considered toxic. (This is also true of the Vip3Aa1 insecticidal protein that was tested.) Further, amino acid sequence comparisons showed no similarities between Vip3Aa19 or Vip3Aa20, on the one hand, and known toxic proteins in protein databases, on the other hand, that would raise a safety concern.

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,* the microorganism from which Vip3Aa proteins are derived, is not considered to be a source of allergenic proteins.
2. Amino acid sequence. A comparison of the amino acid sequence of Vip3Aa19 and 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. Both Vip3Aa19 and Vip3Aa20 insecticidal proteins are digested rapidly in simulated gastric fluid containing pepsin.
4. Glycosylation. Both Vip3Aa19 and Vip3Aa20 were shown not to be glycosylated.

Considering all of the available information on Vip3Aa19 and Vip3Aa20, EPA concludes that the potential for these specific proteins to be food allergens is minimal. Moreover, as further explained below, EPA believes these data and the other submitted data demonstrating a lack of mammalian toxicity at high levels of exposure to Vip3Aa19 and Vip3Aa20 can be extrapolated to cover Vip3Aa proteins more generally.

Vip3Aa is the designation assigned to a closely related group of similar insecticidal proteins isolated from *Bacillus thuringiensis*. The specific variants referred to throughout this document (i.e., Vip3Aa19 and Vip3Aa20) are isolates of Vip3Aa protein. All Vip3Aa proteins (there are 25 known Vip3Aa proteins and there are sequences available for 19 of these) are highly related. Indeed, the amino acid sequence of all the Vip3Aa proteins can only vary up to 5% to be considered a part of the Vip3Aa group. With respect to the 19 Vip3Aa proteins for which sequences are available, they vary by less than 28 amino acids out of the 789 amino acids that make up the protein. This level of sequence similarity makes that group of 19 Vip3Aa protein variants 96% identical overall. The sequence identity between any two individual sequences is even higher. For example, the sequences of the protein variants tested by Syngenta (i.e., Vip3Aa1, Vip3Aa19, and Vip3Aa20) are over 99.7%

identical. Finally, as to the few amino acid differences that do exist between the Vip3Aa variants, these differences do not alter the surrounding sequence, rarely occur as contiguous amino acids, and are often substitutions with similar chemical side groups indicating similar chemical functionality. Therefore, EPA finds that none of the Vip3Aa variants would be expected to have significant amino acid sequence identity with known or putative allergens, which is defined as either 35% identity over an 80 amino acid stretch or identity at the level of eight contiguous amino acid residues.

This conclusion is further supported by EPA's overall safety assessment that includes other considerations such as the source of the trait, digestibility, and glycosylation. As noted above, *Bacillus thuringiensis* (from which the Vip3Aa proteins are derived) is not considered to be a source of allergenic proteins. Furthermore, since all the Vip3Aa proteins have extremely homogenous structural similarities (as explained above), they are highly likely to show similar biochemical characteristics in terms of digestibility and glycosylation. So, as is the case for both Vip3Aa19 and Vip3Aa20, EPA expects that all Vip3Aa proteins will be rapidly digested under simulated gastric conditions and will not be glycosylated. The Vip3Aa proteins were only shown not to be glycosylated in cotton and corn, similarly it is unlikely to be glycosylated in any other crops because in order for a protein to be glycosylated, it needs to contain specific recognition sites for the enzymes involved in glycosylation, and the mechanisms of protein glycosylation are similar in different plants (Lerouge *et al*. 1998). Thus, EPA reasonably expects that because the data on Vip3Aa proteins in cotton and corn demonstrate a lack of protein glycosylation, it will not be glycosylated in any other plants.

Finally, it is also highly relevant here that microbial pesticide products, which are distinct from plant-incorporated protectant pesticide products, containing *Bacillus thuringiensis* and its components (which could include microbially expressed Vip3Aa proteins) are already exempt from the requirement for a tolerance under 40 CFR § 180.1011.

Accordingly, EPA believes that the foregoing supports EPA's reasonable certainty of no harm finding not only for the Vip3Aa19 and Vip3Aa20 insecticidal protein variants, but also for all other closely related members of the Vip3Aa designation as described using the Crickmore classification system (Crickmore *et al*. 2009).

ii. Aggregate Exposures

Pursuant to FFDCA section 408(b)(2)(D)(vi), EPA considers available information concerning aggregate exposures from the pesticide residue in food and all other non-occupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

The Agency has considered available information on the aggregate exposure levels of consumers (and major identifiable subgroups of consumers) to the pesticide chemical residue (i.e., the Vip3Aa proteins) and to other related substances. These considerations include dietary exposure under the

tolerance exemption and all other tolerances or exemptions in effect for the plant-incorporated protectant's chemical residue, and exposure from non-occupational sources. Exposure via the skin or inhalation is not likely since the plant-incorporated protectant is contained within plant cells, which essentially eliminates these exposure routes or reduces these exposure routes to negligible. In addition, even if exposure can occur through inhalation, the potential for Vip3Aa proteins to be allergens is low, as discussed above. Although the allergenicity assessment focuses on potential to be a food allergen, the data also indicate a low potential for Vip3Aa proteins to be inhalation allergens. Exposure *via* residential or lawn use to infants and children is also not expected because the use sites for Vip3Aa proteins are agricultural. Oral exposure, at very low levels, may occur from ingestion of processed products and, theoretically, drinking water. However, oral toxicity testing showed no adverse effects.

iii. Cumulative Effects

Pursuant to FFDCA section 408(b)(2)(D)(v), EPA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Because there is no indication of mammalian toxicity from exposure to Vip3Aa proteins, we conclude that there are no cumulative effects for the Vip3Aa proteins.

iv. Determination of Safety for U.S. Population, Infants, and Children

a. Toxicity and Allergenicity Conclusions

The data submitted and cited regarding potential health effects for Vip3Aa proteins includes the characterization of representative Vip3Aa proteins, as well as the acute oral toxicity studies, amino acid sequence comparisons to known allergens and toxins, and *in vitro* digestibility of the representative Vip3Aa proteins. The results of these studies were used to evaluate human risk, and the validity, completeness, and reliability of the available data from the studies were also considered.

Adequate information was submitted to show that the Vip3Aa test materials derived from microbial cultures were biochemically and functionally equivalent to the proteins produced by the plant-incorporated protectant ingredient in the plants. Microbially produced proteins were used in the studies so that sufficient material for testing was available.

The acute oral toxicity data submitted for the representative Vip3Aa proteins support the prediction that Vip3Aa proteins will be non-toxic to humans. As mentioned above, when proteins are toxic, they are known to act *via* acute mechanisms and at very low dose levels (Sjoblad *et al*. 1992). Since no treatment-related adverse effects were shown to be caused by the representative Vip3Aa proteins, even at relatively high dose levels, Vip3Aa proteins are not considered toxic. Basing this conclusion

on acute oral toxicity data without requiring further toxicity testing or residue data is similar to the Agency position regarding toxicity and the requirement of residue data for the microbial *Bacillus thuringiensis* products from which this plant-incorporated protectant was derived (See 40 CFR § 158.2140). For microbial products, further toxicity testing (Tiers II and III) and residue data are triggered when significant adverse effects are seen in studies such as the acute oral toxicity study. Further studies verify the observed adverse effects and clarify the source of these effects.

Residue chemistry data were not required for a human health effects assessment of the subject plant-incorporated protectant ingredients because of the lack of mammalian toxicity. However, data submitted demonstrated low levels of the representative Vip3Aa proteins in corn and cotton tissues.

Since Vip3Aa isolates are proteins, potential allergenicity is also considered as part of the toxicity assessment. Considering all of the available information, including that (1) Vip3Aa proteins originate from a non-allergenic source; (2) Vip3Aa19 and Vip3Aa20 have no sequence similarities with known allergens; (3) Vip3Aa19 and Vip3Aa20 are not glycosylated; (4) Vip3Aa19 and Vip3Aa20 are rapidly digested in simulated gastric fluid; and (5) the data developed for Vip3Aa19 and Vip3Aa20 can be extrapolated to all Vip3Aa proteins due to the extremely high level of structural similarity that exists between and among Vip3Aa proteins, EPA has concluded that the potential for Vip3Aa proteins to be allergenic is minimal.

Neither available information concerning the dietary consumption patterns of consumers (and major identifiable subgroups of consumers including infants and children) nor safety factors that are generally recognized as appropriate for the use of animal experimentation data were evaluated. The lack of mammalian toxicity at high levels of exposure to representative Vip3Aa proteins, as well as the minimal potential to be a food allergen, demonstrate the safety of Vip3Aa proteins at levels well above possible maximum exposure levels anticipated.

The genetic material necessary for the production of the plant-incorporated protectant active ingredient include the nucleic acids (DNA, RNA) that encode these proteins and regulatory regions. The genetic material (DNA, RNA), necessary for the production of Vip3Aa proteins has been exempted from the requirement of a tolerance under 40 CFR § 174.507 (“Nucleic acids that are part of a plant-incorporated protectant”).

b. Infants and Children Risk Conclusions

FFDCA section 408(b)(2)(C) provides that EPA shall assess the available information about consumption patterns among infants and children, special susceptibility of infants and children to pesticide chemical residues, and the cumulative effects on infants and children of the residues and other substances with a common mechanism of toxicity. In addition, FFDCA section 408(b)(2)(C) also provides that EPA shall apply an additional tenfold margin of safety for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of

the database unless EPA determines that a different margin of safety will be safe for infants and children.

In this instance, based on all the available information, the Agency concludes that there is a finding of no toxicity for Vip3Aa proteins. Thus, there are no threshold effects of concern and, as a result, the provision requiring an additional margin of safety does not apply. Further, the considerations of consumption patterns, special susceptibility, and cumulative effects do not apply.

c. Overall Safety Conclusion

There is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to Vip3Aa proteins. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion because, as discussed above, no toxicity to mammals has been observed, nor any indication of allergenicity potential for Vip3Aa proteins.

v. Other Considerations

a. Endocrine Disruptors

The pesticidal active ingredient is a protein, derived from a source that is not known to exert an influence on the endocrine system. Therefore, the Agency is not requiring information on the endocrine effects of the plant-incorporated protectant at this time.

b. Analytical Method(s)

A validated lateral flow enzyme-linked immunosorbent assay protocol has been provided to the Agency for detecting Vip3Aa in cotton as well as a qualitative ELISA method for detecting Vip3Aa in corn.

c. Codex Maximum Residue Level

No Codex maximum residue level exists for the plant-incorporated protectant, *Bacillus thuringiensis* Vip3Aa proteins and the genetic material necessary for their production in corn and cotton.

vi. Tolerance Exemptions

The data submitted and reviewed for Vip3Aa proteins support the petition for an exemption from the requirement of tolerance for *Bacillus thuringiensis* Vip3Aa proteins when used as plant-incorporated protectants in or on the food and feed commodities of corn and cotton.

vii. Supporting Data

The human health studies submitted to support the safety of Vip3Aa proteins (and Vip3Aa20 in particular) are summarized in Table 4 below.

Table 4. Summary of Vip3Aa Human Health Data (Reviewed in Barsoum and Kough 2008 Unless Otherwise Noted)

Study Type/Title	Summary	MRID No.
Summary of Mammalian Toxicology Data for VIP3A Proteins Produced by VIP3A Cotton Event COT102[f]	Acute oral toxicity in male and female mice was not observed at approximately 3,675 mg VIP3A/kg body weight (the highest dose tested) and the LD_{50} for pure VIP3A protein was >3,675 mg/kg body weight. **Classification: ACCEPTABLE**	457665-02
Acute Oral Toxicity Study with Test Substance VIP3A-0100 Protein in Mice[f]	The test animals (male and female Cr1-1® [ICR] BR mice, 16 each) were quarantined for 9 days and fasted approximately 4 hours prior to dosing. The test material (5,000 mg/kg body weight) was dosed as a suspension of 196 mg/mL in 0.5% weight by volume (w/v) carboxymethyl cellulose (CMC) in deionized water by gavage. The dose volume was 25.5 mL/kg. The control group was treated with 0.5% w/v CMC in the same manner as the test animals. Body weights were recorded prior to dosing and on days 8 and 15 for animals designated to be sacrificed on day 15. The animals were observed for clinical signs of toxicity approximately 1, 2.5, 4, and 6 hours post dosing and at least daily until sacrifice. All animals sacrificed on day 15 had normal body weight gains. Necropsy findings showed no test material related microscopic alterations. In addition, no significant differences considered to be test material related in organ/body weight or organ/brain weight between control and test animals were found. The oral LD_{50} for males, females, and combined was greater than 5,000 mg/kg (or >3,675 mg VIP3A protein/kg body weight). **Classification: ACCEPTABLE**	457665-05
Summary of Mammalian Toxicology Data for the VIP3A and APH4 Proteins Produced by Transgenic VIP3A Cotton Event COT102[f]	The study report is a summary of the results reported in the various reports submitted for consideration of a Section 5 experimental use permit and a Section 3 registration. This volume does not constitute a study in the sense of data collection, but rather a compilation of data and concepts related to risk assessment for the VIP3A protein. The data and information contained in this volume supplement information previously submitted to the Agency in a summary volume titled, "Summary of Mammalian Toxicology Data for the VIP3A and APH4 Proteins Produced by Transgenic VIP3A Cotton Event COT102" (MRID No. 457665-02; Vlachos, 2002; submitted September 24, 2002). Briefly, the VIP3A protein, as found in COT102 cotton, is non-toxic to mammals at the dose given (LD_{50} >3,675 mg VIP3A/	458358-04

[f] Study submitted with experimental use permit (EUP) request and reviewed in memorandum from C. Wozniak, Ph.D. to L. Cole dated March 24, 2004.

Study Type/Title	Summary	MRID No.
	kg body weight), susceptible to degradation in a simulated gastric fluid assay, heat labile, and contains no homology to allergens or toxins when compared to databases of known allergens and toxins. **Classification: NOT APPLICABLE, SUMMARY OF PREVIOUSLY REVIEWED DATA**	
In vitro Digestibility of VIP3A Protein under Simulated Mammalian Gastric Conditions[f]	VIP3A from recombinant maize (field corn) plants was prepared as sample LPPACHA-0199 by extracting protein from the leaves of recombinant corn plants and concentrating the VIP3A by ammonium sulfate precipitation, dialysis of the resulting salt, and lyophilization of the collected protein. Enzyme-linked immunosorbent assay showed VIP3A constituted ~0.36% by weight of the sample and retained insecticidal activity against sensitive lepidopteran species. VIP3A from *E. coli* was prepared as sample VIP3A-0100 in an *E. coli* strain BL21DE3pLysS over-expression system. The synthetic *vip3A(a)* gene was cloned into the inducible over-expression pET-3a® vector. Following collection, purification, dialysis, and lyophilization, the sample was estimated by ELISA to contain ~73.5% VIP3A by weight and it retained its insecticidal activity against sensitive lepidopteran species. For the *in vitro* gastric digestibility study, the reactions were initiated by the addition of 80 µL of LPPACHA-0199 or VIP3A-0100 to 320 µL of simulated gastric fluid containing pepsin incubated at 37°C. Immediately after sample addition, an aliquot was removed and quenched with an equal volume of Laemmli buffer (pH not reported) and inactivated at >75°C for 10 minutes. Additional aliquots were removed and treated as above following 2, 5, 10, 20, 30, and 60 minutes of incubation *via* SDS-PAGE and western blotting. The digestion of VIP3A protein in a simulated gastric environment proceeds at a rapid rate and demonstrates the lability of this protein to conditions typical of a monogastric mammalian stomach. Therefore, results of this study indicate VIP3A protein, whether isolated from recombinant corn plants or from genetically modified *E. coli*, will be rapidly digested in a simulated gastric environment. **Classification: ACCEPTABLE**	458358-05
Vip3A as Expressed in Event MIR162 Maize: Assessment of Amino Acid Sequence Homology with Known Toxins[g]	The purpose of the study was to determine if Event MIR162 Vip3A protein had any significant amino acid sequence homology to known or putative protein toxins. The database identified 32 entries with E values below 6 x 10^{-6}, of which 30 were vegetative insecticidal proteins of *B. thuringiensis* and had E values of 0.0 to 1 x 10^{-10}. Two proteins were identified as rhoptry proteins from *Plasmodium yoelii*, a pathogen that causes malaria in rodents *via* erythrocyte binding and invasion (Ogun and Holder 1996). Despite the pathogenic nature of *P. yoelii*, the low overall sequence similarity between MIR162 Vip3A and the rhoptry proteins (3.9 or 11.4% overall amino acid sequence identity) suggests that the E values are of no biological	468648-08

[f] Study submitted with experimental use permit (EUP) request and reviewed in memorandum from C. Wozniak, Ph.D. to L. Cole dated March 24, 2004.

[g] Study submitted with EUP request (Event MIR162 corn) and reviewed in memorandum from A. Waggoner, through J. Kough, Ph.D., to M. Mendelsohn dated February 8, 2007.

Study Type/Title	Summary	MRID No.
	significance (Doolittle 1990). Furthermore, a global protein alignment (Myers and Miller 1988) demonstrates that there are no more than three contiguous identical amino acids between Vip3A and the rhoptry proteins. Therefore, no relevant similarities between the Event MIR162 Vip3A query sequence and known protein toxins were found. **Classification: ACCEPTABLE**	
Vip3A as Expressed in Event MIR162 Maize: Assessment of Amino Acid Sequence Homology with Known Allergens[g]	The purpose of this study was to determine if Event MIR162 Vip3A had any significant amino acid sequence homology to known or putative protein allergens. No significant sequence homology was found between any sequential MIR162 Vip3A 80-amino acid peptides and any entry in the SBI Allergen Database. No alignments of eight or more contiguous identical amino acids were identified between MIR162 Vip3A and proteins in the SBI Allergen Database. Therefore, no significant amino acid sequence homology was found between the MIR162 Vip3A and any known or putative protein allergens. **Classification: ACCEPTABLE**	468648-09
Analysis of Vip3A or Vip3A-Like Proteins in Six Different Commercial Microbial *Bacillus thuringiensis* Products[h]	The purpose of this study was to determine whether Vip3A or Vip3A-like proteins are detectable and quantifiable in commercial formulations of *Bacillus thuringiensis*-based microbial insecticide products. Enzyme-linked immunosorbent assay and western blot analyses were used to detect and analyze Vip3A or Vip3A-like proteins in the formulations. Vip3A or Vip3A-like proteins were detected in all six commercial products, with concentrations ranging from a low of *ca*. 2.0 µg/g product to a high of *ca*. 209 µg/g. Those products showing the highest protein concentrations were all derived from the *kurstaki* subspecies of *B. thuringiensis*. **Classification: ACCEPTABLE**	470176-13
Single Dose Oral Toxicity Study in Mice	In a 15-day acute oral toxicity study, a single dose of Vip3Aa20 (purity 84%, Lot No. L0749/140/071-085) was administered by gavage in corn oil to groups of five Alpk:AP$_f$CD1 mice/sex/dose at concentrations of 0 to 1,250 mg/kg. No clinical signs of toxicity were observed and no significant treatment-related effects were found on mortality, body weight, food consumption, hematology or clinical chemistry parameters, or organ weight. No significant treatment-related effects were found microscopically. **Classification: ACCEPTABLE**	471378-08
In vitro Digestibility of Vip3Aa20 under Simulated Mammalian Gastric Conditions	No intact Vip3Aa20 insecticidal protein from bacterial- or plant-derived sources was found one minute after incubation in simulated gastric fluid. An immunoreactive Vip3Aa20 polypeptide fragment (~60 kiloDaltons) in the digestion mixture was present at one minute only from the plant-derived source but was not detectable after two minutes of incubation. The study suggests that Vip3Aa20 is readily digested in simulated mammalian gastric	471378-09

[g] Study submitted with EUP request (Event MIR162 corn) and reviewed in memorandum from A. Waggoner, through J. Kough, Ph.D., to M. Mendelsohn dated February 8, 2007.

[h] Study submitted with Section 3 registration request (COT67B x COT102 cotton) and reviewed in memorandum from R. Edelstein, Ph.D., through J. Kough, Ph.D., to A. Reynolds dated February 7, 2008.

Study Type/Title	Summary	MRID No.
	fluid. **Classification: ACCEPTABLE**	
In vitro Digestibility of Vip3Aa20 (MIR162VIP3A-0106) under Simulated Mammalian Intestinal Conditions	No intact Vip3Aa20 protein from bacterial-derived sources was found five minutes after incubation in simulated intestinal fluid. Immunoreactive Vip3Aa20 polypeptide fragments of ~62 kiloDaltons and ~55 kiloDaltons were present in the digestion mixture after five and fifteen minutes of incubation, respectively. The study suggests that intact Vip3Aa20 is readily digested completely or into two smaller polypeptide fragments in simulated mammalian intestinal fluid. **Classification: ACCEPTABLE**	471378-10
Effect of Temperature on Stability of Vip3Aa20 Protein	The study was done to determine the effect of temperature on the stability of the insecticidal protein, Vip3Aa20, by incubating the test material for 30 minutes at 25°C, 37°C, 65°C, or 95°C followed by bioassay against the fall armyworm (*Spodoptera frugiperda*). An additional sample of the test material was incubated at 4°C to determine baseline activity. Heating of *E. coli*-derived Vip3Aa20 at 65°C or 95°C for 30 minutes eliminated the insecticidal activity of the protein. No significant effect on the protein's insecticidal properties was found following incubation for 30 minutes at temperatures ≤ 37°C. **Classification: ACCEPTABLE**	471378-11

2. Human Health Assessment of Phosphomannose Isomerase (PMI)

The phosphomannose isomerase protein expressed in the MIR162 and MIR604 parental events (and the MIR162 maize, *Bt*11 x MIR162 corn, and *Bt*11 x MIR162 x MIR604 corn products) is covered by the exemption from the requirement of a tolerance at 40 CFR § 174.527 ("Phosphomannose isomerase in all plants; exemption from the requirement of a tolerance").

The human health studies submitted to support the safety of PMI are summarized in Table 5 below.

Table 5. Summary of PMI Human Health Data

Study Type/Title	Summary	MRID No.
Phosphomannose Isomerase (Sample PMI-0198): Acute Oral Toxicity in Mice[i]	An acute mouse oral toxicity study was conducted using the *E. coli*-derived PMI. Phosphomannose isomerase was administered to 7 male and 6 female young mice *via* gavage at a dose of 5,050 mg/kg body weight equivalent to *ca*. 3,080 mg pure PMI/kg body weight. No test substance-related mortalities or clinical signs of toxicity occurred during the study. Gross	459344-07

[i] Study submitted with request for a determination of dietary safety from Syngenta and reviewed in memorandum from J. Kough, Ph.D. to M. Mendelsohn dated January 30, 2004.

	necropsy of the remaining mice at study termination revealed no observable abnormalities. The no observed effect level (NOEL) was *ca.* 3,080 mg PMI protein/kg body weight. The oral LD_{50} of PMI-0198 protein for males, females, and combined was greater than 3,080 mg PMI protein/kg body weight. **Classification: ACCEPTABLE – TOXICITY CATEGORY III**	
In vitro Digestibility of PMI Protein under Simulated Mammalian Gastric and Intestinal Conditions[i]	The susceptibility of PMI to proteolytic degradation was evaluated in simulated mammalian gastric fluid containing pepsin and also in simulated mammalian intestinal fluid (SIF) containing pancreatin. Full-length PMI-0198 protein was degraded to undetectable levels in Coomassie blue stained SDS-PAGE gels after incubation in simulated gastric and intestinal fluids. **Classification: ACCEPTABLE**	459344-08
Effects of Temperature on the Stability of Phosphomannose Isomerase[i]	The phosphomannose isomerase enzyme derived from *E. coli* was prepared at 0.44 mg/mL in standard buffer (50mM Tris-HCl, pH 7.0) and incubated at ambient temperature (25°C), 37°C, 55°C, 65°C, and 95°C for 30 minutes. Phosphomannose isomerase enzymatic activity was monitored by measuring nicotinamide adenosine dinucleotide phosphate reduced (NADPH) production using a spectrophotometer. Results indicate that incubation at ambient temperature (25°C) and at 37°C and 55°C for 30 minutes had little effect on PMI. However, incubation at 65°C and 95°C for 30 minutes inactivated PMI. **Classification: ACCEPTABLE**	459344-09
Phosphomannose Isomerase Protein: Assessment of Amino Acid Sequence Homology with Known Toxins[j]	The purpose of this study was to determine if phosphomannose isomerase derived from *Escherichia coli* had significant amino acid sequence homology to known protein toxins. The database identified 186 entries with E values below 0.087. All of these entries were known or putative PMI enzymes (including mannose-6-phosphate isomerase [MPI] and ManA) with no known toxic activity from 126 species, with E values of 0.0–0.067. Therefore, no relevant similarities were found between the *E. coli* PMI query sequence and known protein toxins. **Classification: ACCEPTABLE**	468648-10
Phosphomannose Isomerase: Assessment of Amino Acid Sequence Homology with Known Allergens[j]	The purpose of this study was to determine if phosphomannose isomerase protein derived from *E. coli* had any significant amino acid sequence homology to known or putative protein allergens. No significant sequence homology was found between any sequential PMI 80-amino acid peptides and any entry in the SBI Allergen Database. Screening of PMI amino acid sequence for matches of eight or more contiguous amino acids with the allergen database revealed one alignment, that with the allergen, α-parvalbumin from *Rana species* CH2001. Hilger *et al*. (2002) identified α-parvalbumin as an allergen in an individual who had severe anaphylaxis after eating frog legs of Indonesian origin. This patient's serum was not cross-reactive to related parvalbumins from the common edible frog (*Rana esculenta*). The common amino acid sequence of DLSDKETT occurred at positions 327–334 in PMI, and at positions 77–84 in α-parvalbumin. In order	468648-11

[i] Study submitted with request for a determination of dietary safety from Syngenta and reviewed in memorandum from J. Kough, Ph.D. to M. Mendelsohn dated January 30, 2004.

[j] Study submitted with EUP request (Event MIR162 corn) and reviewed in memorandum from A. Waggoner, through J. Kough, Ph.D., to M. Mendelsohn dated February 8, 2007.

	to determine if the IgE antibodies present in this patient's serum recognized PMI, serum obtained from the one identified person with IgE-mediated allergy to α-parvalbumin from *Rana species* CH2001 was not cross-reactive with PMI overexpressed in *E. coli* (PMI-098; containing 61% weight by weight [w/w] PMI protein and having PMI enzymatic activity). Therefore, it is concluded that this 8-amino acid sequence identity with α-parvalbumin from *Rana species* CH2001 was not biologically relevant, and that there is no evidence that *E. coli*-derived PMI has significant amino acid sequence homology to any known or putative allergenic proteins. EPA previously reviewed this study and concurred with the study author's conclusion. **Classification: ACCEPTABLE**	

3. Human Health Assessment of Cry1Ab and Phosphinothricin Acetyltransferase (Expressed in *Bt*11 x MIR162 and *Bt*11 x MIR162 x MIR604) and mCry3A (expressed in *Bt*11 x MIR162 x MIR604)

As mentioned previously, EPA granted registration for *Bacillus thuringiensis* subspecies *kurstaki* strain HD-1 Cry1Ab protein and the genetic material necessary for its production in Event MON 810 corn (EPA Reg. No. 524-489) in 1996. The Agency concluded that there were no adverse effects on human health from the use of the Cry1Ab insecticidal protein expressed in corn. Subsequently, an exemption from the requirement for a food tolerance was established when Cry1Ab insecticidal protein is used as a plant-incorporated protectant under 40 CFR § 174.511. Syngenta bridged data from MON 810 and provided additional product characterization data to register Cry1Ab expressed in Event *Bt*11 corn (EPA Reg. No. 67979-1 for field corn; EPA Reg. No. 65268-1 for sweet corn). The phosphinothricin acetyltransferase (PAT) protein is also expressed with Cry1Ab in *Bt*11 corn, as a PIP inert ingredient, and was granted an exemption from the requirement of a tolerance in all food commodities under 40 CFR § 174.522. The toxicological and allergenicity data supporting the registration of *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production (*via* elements of plasmid vector pZO1502) in corn, which includes the submitted study titles, conclusions, and their MRID Numbers, are found in the 2001 *Bt* Crops Reassessment (U.S. EPA 2001). EPA determined that the human health data previously submitted for Cry1Ab produced in MON 810 is applicable to Cry1Ab produced in Event *Bt*11 (U.S. EPA 2001).

The Agency established a permanent exemption from the requirement of a tolerance for modified Cry3A (mCry3A) protein under 40 CFR § 174.505 and issued a Section 3 registration for Event MIR604 corn (EPA Reg. No. 67979-5) on October 3, 2006. The toxicological and allergenicity data supporting the registration of *Bacillus thuringiensis* mCry3A protein and the genetic material necessary for its production (*via* elements of plasmid vector pZM26) in Event MIR604 corn, which includes the submitted study titles, conclusions, and their MRID Numbers, are found in the mCry3A BRAD (U.S. EPA 2007).

Based on data currently available and the conclusion that no synergistic action or interaction of the above-mentioned proteins (to include Vip3Aa20 and PMI) is known or expected to occur, the existing exemptions from the requirement of a food tolerance for Cry1Ab and mCry3A (and Vip3Aa) insecticidal proteins, as well as those for the PAT (and PMI) inert proteins, are amended to support the addition of *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn, when used as plant-incorporated protectants.

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C. Environmental Hazard Assessment

1. Environmental Risk Assessment for MIR162 (Lepidopteran Active) Maize

i. Tiered Hazard and Risk Assessment Process

To minimize data requirements and avoid unnecessary tests, risk assessments are structured such that risk is determined first from estimates of hazard under "worst-case" exposure conditions. A lack of adverse effects under these conditions would provide enough confidence that there is no risk and no further data would be needed. Hence, such screening tests conducted early in an investigation tend to be broad in scope but relatively simple in design, and can be used to demonstrate acceptable risk under most conceivable conditions. When screening studies suggest potentially unacceptable risk, additional studies are designed to assess risk under more realistic field exposure conditions. These later tests are more complex than earlier screening studies. Use of this "tiered" testing framework saves valuable time and resources by organizing the studies in a cohesive and coherent manner and eliminating unnecessary lines of investigation. Lower tier, high-dose screening studies also allow tighter control over experimental variables and exposure conditions, resulting in a greater ability to produce statistically reliable results at relatively low cost[a].

Tiered tests are designed to first represent unrealistic worst-case scenarios and ONLY progress to real-world field scenarios if the earlier tiered tests fail to indicate adequate certainty of acceptable risk. Screening (Tier I) non-target organism hazard tests are conducted at exposure concentrations several times higher than the highest concentrations expected to occur under realistic field exposure scenarios. This has allowed an endpoint of 50% mortality to be used as a trigger for additional higher tier testing. Less than 50% mortality under these conditions of extreme exposure suggest that population effects are likely to be negligible given realistic field exposure scenarios.

The Environmental Protection Agency (EPA) uses a tiered (Tiers I–IV) testing system to assess the toxicity of a plant-incorporated protectant (PIP) to representative non-target organisms that could be exposed to the toxin in the field environment. Tier I high-dose studies reflect a screening approach to testing designed to maximize any toxic effects of the test substance on the test (non-target) organism. The screening tests evaluate single species in a laboratory setting with mortality as the endpoint.

[a] Non-target invertebrate hazard tests often are conducted at exposure concentrations several times higher than the maximum concentrations expected to occur under realistic exposure scenarios. This has customarily allowed an endpoint of 50% mortality to be used as a trigger for additional higher tier testing. Lower levels of mortality under these conditions of extreme exposure suggest that population effects are likely to be negligible given realistic exposure scenarios. Thus, it follows that the observed proportion of responding individuals can be compared to a 50% effect to determine if the observed proportion is significantly lower than 50%. For example, using a binomial approach, a sample size of 30 individuals is sufficient to allow a treatment effect of 30% to be differentiated from a 50% effect with 95% confidence using a one-sided Z test. A one-sided test is appropriate because only effects of less than 50% indicate that further experiments are not needed to evaluate risk.

Tiers II–IV generally encompass definitive hazard level determinations, longer term greenhouse or field testing, and are implemented when unacceptable effects are seen at the Tier I screening level.

Testing methods, which utilize the tiered approach, were last published by the EPA as Harmonized Office of Prevention, Pesticides, and Toxic Substances (OPPTS) Testing Guidelines, Series 850 and 885 (EPA 712-C-96-280, February 1996)[b]. These guidelines apply to microbes and microbial toxins when used as pesticides (as defined in 40 Code of Federal Regulations [CFR] § 152.20), including those that are naturally occurring, and those that are strain improved either by natural selection or by deliberate genetic manipulation. Therefore, PIPs containing microbial toxins are also covered by these testing guidelines.

The Tier I screening maximum hazard dose (MHD) approach to environmental hazard assessment is based on some factor (whenever possible >10) times the maximum amount of active ingredient expected to be available to terrestrial and aquatic non-target organisms in the environment, or the Estimated Environmental Concentration (EEC)[c]. Tier I tests serve to identify potential hazards and are conducted in the laboratory at high dose levels, which increase the statistical power to test the hypotheses. Elevated test doses, therefore, add certainty to the assessment, and such tests can be well standardized. The Guidelines call for initial screening testing of a single group or several groups of test animals at the maximum hazard dose level. The Guidelines call for testing of one treatment group of at least thirty animals or three groups of ten test animals at the screening test concentration. The Guidelines further state that the duration of all Tier I tests should be approximately 30 days. Some test species, notably non-target insects, may be difficult to culture and the suggested test duration has been adjusted accordingly. Control and treated insects should be observed for at least 30 days, or in cases where an insect species cannot be cultured for 30 days, until negative control mortality rises above 20%.

Failing the Tier I (10x EEC) screening at the MHD does not necessarily indicate the presence of an unacceptable risk in the field, but it triggers the need for additional testing[d]. A less than 50% mortality effect at the MHD is taken to indicate minimal risk. However, greater than 50% mortality does not necessarily indicate the existence of unacceptable risk in the field, but it does trigger the

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[b] General OPPTS Harmonized Testing Guidelines available from: *http://www.epa.gov/opptsfrs/home/guidelin.htm*. Series 850 Testing Guidelines available from: *http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/*. Series 885 Testing Guidelines available from: *http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/885_Microbial_Pesticide_Test_Guidelines/Series/*.

[c] The dose margin can be less than 10x where uncertainty in the system is low or where high concentrations of test material are not possible to achieve due to test organism feeding habits or other factors. High-dose testing also may not be necessary where many species are tested or tests are very sensitive, although the test concentration used must exceed 1x EEC.

[d] It is notable that the 10x EEC MHD testing approach is not equivalent to what is commonly known as "testing at a 10x safety factor," where any adverse effect is considered significant. Tier I screen testing is not "safety factor testing." In a "10x safety factor" test, any adverse effect noted is a "level of concern," whereas in the EPA environmental risk assessment scenario any adverse effect is viewed as a concern only at 1x the field exposure.

need to collect additional dose-response information and a refinement of the exposure estimation before deciding if the risk is acceptable or unacceptable. Where potential hazards are detected in Tier I testing (i.e., mortality is greater than 50%), additional information at lower test doses is required, which can serve to confirm whether any effect might still be detected at more realistic field (1x EEC) concentrations and routes of exposure[e].

When screening tests indicate a need for additional data, the OPPTS Harmonized Guidelines call for testing at incrementally lower doses in order to establish a definitive LD_{50} (i.e., dose that will kill 50% of the test organisms within a designated period) and to quantify the hazard. In the definitive testing, the number of doses and test organisms evaluated must be sufficient to determine an LD_{50} value and, when necessary, the Lowest Observed Adverse Effect Concentration (LOAEC), No Observed Adverse Effect Level (NOAEL), or reproductive and behavioral effects such as feeding inhibition, weight loss, etc. In the final analysis, a risk assessment is made by comparing the LOAEC to the EEC; when the EEC is lower than the LOAEC, a no risk conclusion is made. These tests offer greater environmental realism, but they may have lower statistical power. Appropriate statistical methods, and appropriate statistical power, must be employed to evaluate the data from the definitive tests. Higher levels of replication, test species numbers, and/or repetition are needed to enhance statistical power in these circumstances.

Data that shows less than 50% mortality at the maximum hazard dosage level (i.e., LC_{50}, ED_{50}, or LD_{50} >10x EEC) is sufficient to evaluate adverse effects, making lower field exposure dose definitive testing unnecessary. It is also notable that the recommended >10x EEC maximum hazard dose level is a highly conservative factor. The published EPA Level of Concern (LOC) is 50% mortality at 5x EEC (U.S. EPA 1998)[f].

Validation: The tiered hazard assessment approach was developed for the EPA by the American Institute of Biological Sciences (AIBS) and confirmed in 1996 as an acceptable method of environmental hazard assessment by a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) on microbial pesticides and microbial toxins. The December 9, 1999 SAP agreed that the Tiered approach was suitable for use with PIPs; however, this panel recommended that, for PIPs with insecticidal properties, additional testing of beneficial invertebrates closely related to target species and/or likely to be present in genetically modified (GM) crop fields

[e] The 1x EEC test dose is based on plant tissue content and is considered the highest dose in a worst-case scenario (sometimes referred to as the Highest Estimated Environmental Concentration or HEEC). This 1x EEC is still much greater than any amount which any given non-target organism may be ingesting in the field because most non-target organisms do not ingest plant tissue.

[f] The established peer and EPA Science Board reviewed guidance on screening test levels of concern is 50% mortality at 5x environmental concentration for terrestrial and 10x for aquatic species. The appropriate endpoints in high-dose limit/screening testing are based on mortality of the treated, as compared to the untreated (control) non-target organisms. A single group of 30 test animals may be tested at the maximum hazard dose.

should be conducted. Testing of *Bacillus thuringiensis* (*Bt*) Cry proteins on species not closely related to the target insect pest was not recommended, although it is still performed to fulfill the published EPA non-target species data requirements. In October 2000, another SAP also recommended that field testing should be used to evaluate population-level effects on non-target organisms. The August 2002 SAP, and some public comments, generally agreed with this approach, with the additional recommendation that indicator organisms should be selected on the basis of potential for field exposure to the subject protein (U.S. EPA 2000, 2001a, 2002, and 2004).

Chronic studies: Since delayed adverse effects and/or accumulation of toxins through the food chain are not expected to result from exposure to proteins, protein toxins are not routinely tested for chronic effects on non-target organisms. However, the 30-day test duration requirement does amount to subchronic testing when performed at field exposure test doses. Proteins do not bioaccumulate. The biological nature of proteins makes them readily susceptible to metabolic, microbial, and abiotic degradation once they are ingested or excreted into the environment. Although there are reports that some proteins (Cry proteins) bind to soil particles, it has also been shown that these proteins are degraded rapidly by soil microbial flora upon elution from soil particles.

Conclusion: The tiered approach to test guidelines ensures, to the greatest extent possible, that the Agency requires the minimum amount of data needed to make scientifically sound regulatory decisions. The EPA believes that maximum hazard dose Tier I screening testing presents a reasonable approach for evaluating hazards related to the use of biological pesticides and for identifying negative results with a high degree of confidence. The Agency expects that Tier I testing for short-term hazard assessment will be sufficient for most studies submitted in support of PIP registrations. However, if long-range adverse effects must be ascertained, then higher tier, longer term field testing will be required. As noted above, the October 2000 SAP and the National Academy of Sciences (NAS 2000) recommended testing non-target organisms directly in the field. This approach, with an emphasis on testing invertebrates found in corn fields, was also recommended by the August 2002 SAP and was supported by several public comments. Based on these recommendations and due to the lack of baseline data on the potential for long-term environmental effects from the cultivation of PIP-producing plants, the Agency has required long-term field studies on invertebrate populations/communities and Cry protein accumulation in soils as conditions of past PIP registrations.

Since the commercialization of *Bt* crops, the number of field studies published in scientific literature in combination with the post-registration field studies submitted to the Agency has accumulated to a level where empirical conclusions can be made. As a result, the issue of long-range effects of cultivation of these Cry proteins on the invertebrate community structure in *Bt* crop fields has since been adequately addressed. Specifically, a meta-analysis[g] of the data collected from 42 field studies

[g] This research was funded by EPA grant CR-832147-01. The *Bt* crop non-target effects database can be found on the National Center for Ecological Analysis and Synthesis (NCEAS) Web Site: *http://delphi.nceas.ucsb.edu/btcrops/*.

indicated that non-target invertebrates are generally more abundant in *Bt* cotton and *Bt* maize fields than in non-transgenic fields managed with insecticides (Marvier *et al*. 2007). In addition, a comprehensive review of short- and long-term field studies on the effects of invertebrate populations in *Bt* corn and cotton fields indicated that no unreasonable adverse effects are taking place as a result of wide-scale *Bt* crop cultivation (Sanvido *et al*. 2007). Another review of field tests published to date concluded that the large-scale studies in commercial *Bt* cotton have not revealed any unexpected non-target effects other than subtle shifts in the arthropod community caused by the effective control of the target pests (Romeis *et al*. 2006). Slight reductions in some invertebrate predator populations are an inevitable result of all pest management practices, which result in reductions in the abundance of the pests as prey.

Overall, the Agency is in agreement with the conclusions of these studies and collectively, these results provide extensive data to support that *Bt* crops have not caused long-term environmental effects, on a population level, to organisms not targeted by *Bt* proteins. Based on these considerations, regulatory testing of the specialist predators and parasitoids of target pests may eventually be considered unnecessary.

ii. Environmental Exposure Assessment

The EPA risk assessment is centered only on adverse effects at the field exposure rates (1x EEC), and not on adverse effects at greater concentrations. Although it is recommended that non-target testing be conducted at a test dose 10x the EEC whenever possible, the test dose margin can be less than 10x where uncertainty in the system is low or where high concentrations of test material are not possible to achieve due to test organism feeding habits. High-dose testing also may not be necessary where many species are tested or tests are very sensitive, although the concentration used must exceed 1x EEC. It is important to note that Tier I screen testing is not "safety factor testing." In a traditional "10x safety factor" test, any adverse effect noted is a "level of concern," whereas in the EPA environmental risk assessment scenario any adverse effect is viewed as a concern only at 1x the field exposure.

For the purposes of the non-target organism (NTO) studies submitted in support of Event MIR162 maize, the test material dose levels were based on the estimated concentration of Vip3Aa insecticidal protein expressed in the tissue(s) that NTOs would most likely be exposed to in the environment (see Waggoner and Kough [2007] and Barsoum and Kough [2008] for protein expression levels). The Agency has determined that the NTOs most likely to be exposed to the Vip3Aa insecticidal protein in transgenic corn fields were beneficial insects feeding on corn pollen. Consequently, test material dose levels were based on the maximum level of measured protein expression in pollen (47.85 micrograms [µg]/gram [g] fresh weight [fwt] for Vip3Aa20). The principal route of Vip3Aa20 insecticidal protein exposure for soil-dwelling organisms (such as collembola, earthworms, and/or rove beetles) is assumed to be from decomposing plant tissue and plant exudates in soil.

Consequently, the dose levels of the test material were based on the maximum level of estimated protein expression in the soil environment.

iii. Non-Target Wildlife Hazard Assessment for Event MIR162 Maize

Two separate SAP reports (October 2000 and August 2002) recommended that non-target testing of *Bt* Cry proteins should focus on invertebrate species exposed to the crop being registered. Following SAP recommendations, the EPA determined that non-target organisms with the greatest exposure potential to Cry protein in transgenic corn fields are beneficial insects, which feed on corn pollen and nectar, and soil invertebrates, particularly Lepidopteran species. The Agency recommended using this same approach for testing the effects of Vip3Aa insecticidal protein in Event MIR162 maize. Therefore, toxicity testing using the maximum hazard dose on representative beneficial organisms from several taxa was performed in support of the Section 3 FIFRA maize registration. The toxicity of the Vip3Aa insecticidal protein has been evaluated on several species of invertebrates including the lady beetle, green lacewing, minute pirate bug, rove beetle, collembola, daphnia, honey bee, and earthworm. In addition, reproductive and developmental observations were examined in the collembola, rove beetle, and honey bee studies.

Vip3Aa20 protein in Event MIR162 maize is very species-specific in its insecticidal activity, conferring toxic effects on black cutworm, fall armyworm, beet armyworm, tobacco budworm, and corn earworm. Despite the October 2000 and August 2002 SAP's recommendations against testing of non-target species not related to susceptible target pests, EPA has completed a risk assessment on a range of non-target wildlife to comply with the Agency's published non-target data requirements. In the absence of PIP-specific risk assessment guidance, EPA requires applicants for PIP registrations to meet the 40 CFR Part 158 data requirements for microbial toxins. These requirements include tests on birds, mammals, plants, and aquatic species. In addition, earthworm and springtail studies were bridged to Vip3Aa19 insecticidal protein data and a rove beetle study was voluntarily submitted to the Agency to ascertain the potential effects of Vip3Aa20 insecticidal protein on beneficial decomposer species.

The October 2000 SAP recommended that while actual plant material is the preferred test material, bacterial-derived protein is also a valid test substance, particularly in scenarios where test animals do not normally consume plant tissue and where large amounts of Cry protein (i.e., Cry protein concentrations that exceed levels present in plant tissue) are needed for maximum hazard dose testing. For Vip3Aa19 insecticidal protein, an insect feeding study compared the relative potency of plant-derived Vip3Aa19 insecticidal protein in Event Pacha corn and Event COT102 cotton to the microbially derived Vip3Aa19 insecticidal protein. For Vip3Aa20 insecticidal protein, an insect feeding study compared the relative potency of plant-derived Vip3Aa20 insecticidal protein in Event MIR162 maize to the microbially derived Vip3Aa20 insecticidal protein. Results from both studies indicated that plant-derived protein was similar in toxicity to the microbially derived protein (Master Record Identification [MRID] Number [No.] 458358-12; Wozniak 2004 and MRID No. 471378-01; Barsoum and Kough 2008). These data indicate that the microbially derived protein for this event is

substantially equivalent to the plant-derived proteins expressed in corn plants, based on the similar insecticidal activity, for studying any potential toxicity on NTOs for the purposes of the environmental risk assessment.

Specifically for Vip3Aa insecticidal protein toxicity tests, Event MIR162 maize expresses the same *vip3A(a)* gene expressed in Event Pacha corn and Event COT102 cotton (although the gene sequences of Event MIR162 maize and Event Pacha corn/Event COT102 cotton are distinguished by quaternary numerical ranks 20 and 19, respectfully) and the expression level of pollen of Event Pacha corn is much higher than that of Event MIR162 maize and Event COT102 cotton. In support of the Event MIR162 maize registration, test substances used in the submitted environmental effects studies included the following: bacterial-produced, purified Vip3Aa20, Vip3Aa19, and Vip3Aa1 insecticidal proteins; plant-expressed Vip3Aa19 insecticidal protein as expressed in Event Pacha corn grain, pollen, and leaves; and plant-expressed Vip3Aa20 insecticidal protein as expressed in Event MIR162 maize grain. The individual results for each study on environmental effects for Vip3Aa are summarized in Table 1. The results are also presented in a more descriptive format in subsequent sections of this Environmental Hazard Assessment chapter. For all events, full reviews of each study can be found in the individual Data Evaluation Reports.

Table 1. Summary of Environmental Effects Studies and Waiver Justifications for Event MIR162 Maize Submitted to Comply with Data Requirements Published in 40 CFR § 158.2150(d)

Data Requirement	OPPTS Guideline	Test Substance	Results Summary and Classification	MRID No.
Avian dietary testing, broiler chicken, *Gallus domesticus*	885.4050	Vip3Aa20 maize grain (Event MIR162 maize)	A 44-day dietary study showed no adverse affects to broiler chickens when fed a diet composed of starter, grower, and finisher diets of Event MIR162 maize grain. The average concentration of the transgenic Event MIR162 maize grain was 14.7 µg Vip3Aa20/g grain. There were no treatment-related differences for mortality, body weight, feed conversion ratio, carcass yield, or clinical chemistry parameters. The diet containing Vip3Aa20 had no deleterious effects on broiler performance or carcass yield. Therefore, the no observed effect concentration (NOEC) was 14.7 µg Vip3Aa20/g feed and the 44-day LC_{50} for broilers was greater than 14.7 µg Vip3Aa20/g MIR162 feed. **Classification: Acceptable**	471378-12
Avian injection testing	885.4100	N/A	**Acceptable waiver rationale**	N/A
Avian oral testing, bobwhite quail, *Colinus virginianus*	850.2100	Microbial Vip3Aa1 (VIP3A-0198)	A 14-day study showed no adverse effects to bobwhite quail from VIP3A-0198, after a single oral dose *via* gavage. The no observed effect level (NOEL) was 400 milligrams (mg) Vip3Aa1/kilogram (kg) and the LD_{50} was >400 mg	457665-08

Data Requirement	OPPTS Guideline	Test Substance	Results Summary and Classification	MRID No.
			Vip3Aa1/kg bird body weight. **Classification: Acceptable**	
Wild mammal testing	885.4150	N/A	**Acceptable bridging rationale to acute oral toxicity test on mice (MRID No. 471378-08).**	N/A
Freshwater fish testing, channel catfish, *Ictalurus punctatus*	885.4200	Vip3Aa19 corn grain (FFPACHA-0100)	A 30-day study showed no adverse effects on juvenile catfish after exposure to Vip3Aa19 protein from Event Pacha corn grain. Therefore, the NOEC was 7.1 µg Vip3Aa19/g diet and the LC_{50} was greater than 7.1 µg Vip3Aa19/g diet consisting of fish feed made from Event Pacha corn grain. **Classification: Acceptable**	470176-24
Freshwater aquatic invertebrate testing, water flea, *Daphnia magna*	885.4240	Vip3Aa19 corn pollen (PHOPACHA-0199)	In a 48-hour static renewal limit bioassay, Vip3Aa19 corn pollen (containing 10.1 µg Vip3Aa19 protein/Liter) had no adverse effects on the survival of *Daphnia magna* when suspended in 120 mg pollen/Liter (L). The LC_{50} was greater than 10.1 µg Vip3Aa19 protein/L. **Classification: Unacceptable.** The Series 885 Guidelines call for a 21-day study. The submitted 48-hour acute study is inadequate.	457921-01
Estuarine and marine animal testing	885.4280	N/A	**Acceptable waiver rationale**	N/A
Non-target plant testing	885.4300	N/A	**Acceptable waiver rationale**	N/A
Non-target insect testing, minute pirate bug/insidious flower bug, *Orius insidiosus*	885.4340	Microbial Vip3Aa19 (VIP3A-0104)	*Orius insidiosus* nymphs were fed a meat-based diet containing 7.25 mg microbially derived Vip3Aa19 protein/g diet and showed no adverse effects after 21 days. The NOEC was 7.25 mg Vip3Aa19 protein/g diet and the LC_{50} was greater than 7.25 mg Vip3Aa19 protein/g diet. **Classification: Acceptable**	468648-14
Non-target insect testing, pink-spotted lady beetle, *Coleomegilla maculata*	885.4340	Vip3Aa19 corn pollen (PHOPACHA-0100)	*Coleomegilla maculata* adults were fed a diet containing 5.0% Vip3Aa19 corn pollen (containing 144.8 µg Vip3Aa19 protein/g pollen) for 21 days with no adverse effects observed. The NOEC was 7.24 µg Vip3Aa19 protein/g diet and the LC_{50} was greater than 7.24 µg Vip3Aa19/g diet. **Classification: Acceptable**	457665-09
Non-target insect testing, seven-spotted ladybird beetle, *Coccinella septempunctata*	885.4340	Microbial Vip3Aa19 (VIP3A-0204)	*Coccinella septempunctata* adults were fed a 50% sucrose diet containing 7.25 mg Vip3Aa19/g diet and showed no adverse effects after 15 days. The NOEC was 7.25 mg Vip3Aa19 protein/g diet and the LC_{50} was greater than 7.25 mg Vip3Aa19 protein/g diet.	468808-02

Data Requirement	OPPTS Guideline	Test Substance	Results Summary and Classification	MRID No.
			Classification: Acceptable	
Non-target insect testing, green lacewing, *Chrysoperla carnea*	885.4340	Microbial Vip3Aa19 (VIP3A-0104)	*Chrysoperla carnea* larvae were fed a meat-based diet containing 7.25 mg Vip3Aa19 protein/g diet and showed no adverse effects. The NOEC was 7.25 mg Vip3Aa19 protein/g diet and the LC_{50} was greater than 7.25 mg Vip3Aa19 protein/g diet at day 14 when the control mortality reached 20%. There were no statistically significant differences between the VIP3A-0104 group and the negative control group. **Classification: Acceptable**	468848-15
Non-target insect testing, rove beetle, *Aleochara bilineata*	885.4340	Microbial Vip3Aa20 (MIR162VIP3A-0106)	*Aleochara bilineata* adults were fed a meat diet containing 595.3 µg Vip3Aa20 protein/g diet for 35 days. Reproductive effects were also assessed by counting the number of second-generation adult beetles emerging from parasitized pupae of the onion fly (*Delia antique).* There were no differences noted between the treatment and negative control groups. The NOEC was 595.3 µg Vip3Aa20 protein/g diet and the LC_{50} was greater than 595.3 µg Vip3Aa20 protein/g diet. **Classification: Acceptable**	471378-13
Non-target insect testing, collembola, *Folsomia candida*	885.4340	Vip3Aa19 corn leaves (LLPACHA-0100)	Collembola were fed a diet containing 50% yeast and 50% leaf tissue for 28 days. No statistically significant effects on survival or reproduction were found among the test and negative control groups. The NOEC was 43.2 µg Vip3Aa19 protein/g diet and the LC_{50} was greater than 43.2 µg Vip3Aa19 protein/g diet. **Classification: Acceptable**	458358-10
Honeybee testing, honeybee larvae, *Apis mellifera*	885.4380	Microbial Vip3Aa20 (MIR162VIP3A-0106)	Honeybees were exposed, *via* oral ingestion, to microbially derived Vip3Aa20 test material in a sucrose solution using in-hive commercial bee feeders. The treatments consisted of 71.4, 285.7, and 714.3 mg MIR162VIP3A-0106/L of sucrose solution, which corresponded to 50, 200, and 500 µg Vip3Aa20 protein/g of solution. The doses represent approximately 1x, 4x, and 10x the highest concentration of Vip3Aa20 in pollen of Event MIR162 maize. The test consisted of a single application of one liter of each solution (including a positive and negative control) per hive and the hives were observed for 24 days for percent	471479-01

Data Requirement	OPPTS Guideline	Test Substance	Results Summary and Classification	MRID No.
			successful brood development to adults and colony conditions. These results indicate direct and incidental ingestion of Vip3Aa20 insecticidal protein did not adversely affect brood development, exposed worker bees, or the hive condition. Therefore, the NOEL was 500 µg Vip3Aa20 protein/g of sucrose solution and the LD_{50} was greater than 500 µg Vip3Aa20 protein/g of sucrose solution. **Classification: Acceptable**	
Earthworm toxicity, earthworm, *Eisenia foetida*	OECD Guideline 207, 850.6200	Vip3Aa19 corn leaves (LPPACHA-0199)	Adult earthworms were exposed to artificial soil containing 3.60 mg Vip3Aa19 protein/kg soil for 14 days. No mortality or differences in body weights were observed in the test group. The NOEC was 3.60 mg Vip3Aa19 protein/kg dry soil and the LC_{50} was greater than 3.60 mg Vip3Aa19 protein/kg dry soil. **Classification: Acceptable**	457921-02
Soil fate and degradation	885.5200	Vip3Aa19 corn leaves (LPPACHA-0199)	Results of this degradation study indicate that the DT_{50} of 16 mg Vip3Aa19 protein/g of soil ranges from 6 to 12.6 days. Therefore, the Vip3Aa19 insecticidal protein is not likely to persist or accumulate in various types of soil. **Classification: Acceptable**	470176-30

a. Non-Target Wildlife Study Summaries for MIR162 Expressing Vip3Aa

Avian Species

Published data and studies on file at EPA show that consumption of *Bt* plants has no measurable deleterious effects on avian species. However, to comply with published data requirements, the following studies were submitted to EPA in support of registration for Vip3Aa protein as expressed in Event MIR162 maize. The broiler chicken study was not conducted in compliance with 40 CFR Part 160, but was conducted according to accepted scientific methods, while the bobwhite quail study was compliant with Good Laboratory Practice (GLP) Standards. When considered together, these studies meet EPA data requirements for avian species risk assessment.

i. Broiler Chicken (MRID No. 471378-12)

For the first 44 days of life, commercial broiler chickens (*Gallus domesticus*) were fed a prepared diet based on transgenic Event MIR162 maize grain containing Vip3Aa20 insecticidal protein, grain from near-isogenic, non-transgenic corn, or grain from locally grown reference corn. The mean Vip3Aa20 concentrations in the test material starter, grower, and finisher diets were 17.59 ± 9.11,

12.26 ± 1.14, and 15.72 ± 1.14 µg/g fresh weight, respectively. Therefore, the average exposure during the study was 14.7 µg/g diet. There were no treatment-related differences for mortality, body weight, feed conversion ratio, carcass yield, or clinical chemistry parameters. The diet containing Vip3Aa20 had no deleterious effects on broiler performance or carcass yield. Therefore, the NOEC was 14.7 µg Vip3Aa20/g feed and the 44-day LC_{50} for broilers was greater than 14.7 µg Vip3Aa20/g MIR162 feed.

Conclusions/Recommendations: No adverse effects were observed on *Gallus domesticus* after a 44-day chronic dietary study after exposure to Event MIR162 maize grain expressing Vip3Aa20 insecticidal protein. The NOEC was 14.7 µg Vip3Aa20/g feed and the LC_{50} for broilers was greater than 14.7 µg Vip3Aa20/g MIR162 feed. Based on the results of the study, the data requirement for avian dietary toxicity is satisfied.

ii. Bobwhite Quail (MRID No. 457665-08)

Five male and five female (*Colinus virginianus*) quails were administered a single oral dose of 2,000 mg VIP3A-0198/kg *via* gelatin capsules. The VIP3A-0198 test substance (microbially derived protein) represented 400 mg Vip3Aa1/kg body weight. No mortalities occurred during the study period. There were no clinical signs of toxicity in any birds during the study. There were no statistically significant changes in body weights after dosing. Additionally, gross pathological examinations of all birds at study termination revealed no abnormalities. The results indicate that the NOEL was 400 mg Vip3Aa1/kg and the LD_{50} was greater than 400 mg Vip3Aa1/kg body weight for bobwhite quail for 14 days.

Conclusions/Recommendations: No adverse effects or mortalities were found after a 14-day acute oral study after exposure to the test substance (VIP3A-0198, microbially derived containing Vip3Aa1). The NOEL was 400 mg Vip3Aa1/kg and the LD_{50} was greater than 400 mg Vip3Aa1/kg body weight for bobwhite quail for 14 days. This study was previously reviewed and found acceptable (Waggoner and Vaituzis 2008a).

Wild Mammalian Species

Mammalian wildlife exposure to Vip3Aa insecticidal protein is considered likely; however, mammalian toxicology information gathered to date on *Bt* Cry and Vip proteins does not show a hazard to wild mammals. In addition, an acute oral toxicity study was submitted to EPA in support of the MIR162 maize registration and indicated no toxicity was seen when rodents were exposed to microbially derived Vip3Aa20 (MIR162VIP3A-0106)insecticidal protein at the maximum hazard dose level (MRID No. 471378-08; Barsoum and Kough 2008). Therefore, no hazard to mammalian wildlife is anticipated from MIR162 maize expressing Vip3Aa20 protein and data on wild mammal testing is not required for this registration.

Aquatic Species

There is no reported toxicity to aquatic organisms from exposure to anti-coleopteran Cry proteins in *Bt* plants. However, a published laboratory study with lepidopteran-active Cry proteins has revealed that the leaf shredding (caddis fly) trichopteran, *Lepidostoma liba*, had 50% lower growth rate when fed *Bt* corn litter (Rosi-Marshall *et al*. 2007). Two previous field study reports by the same authors did not find adverse effects on headwater stream invertebrates. The Agency's position on this matter is that until Tier III and Tier IV field studies are performed, there is not enough information to assert that sufficient corn plant litter enters streams to cause unreasonable adverse effects on stream invertebrate populations or communities (see section II(C)(1)(i) – "Tiered Hazard and Risk Assessment Process"). Two years ago, Iowa State University and University of Maryland received research grants to study the effects of *Bt* corn cultivation on streams and to develop methods for aquatic hazard assessment. The results of these studies are pending. When the study reports are reviewed, the Agency will respond with action commensurate with the outcome of the studies. Therefore, the Agency's current position is that there is no evidence to conclude that there is sufficient aquatic exposure to Cry proteins in corn plant litter to result in adverse effects on stream invertebrate populations or communities.

Farmed fish may be exposed to *Bt* protein in fish feed. However, *Bt* protein activity is generally destroyed during typical fish food manufacturing processes due to protein degradation from the high temperatures. Consequently, exposure of farmed fish to active *Bt* proteins is not expected. Overall, aquatic animal exposure to *Bt* crops is extremely small.

i. Freshwater Fish – Channel Catfish (MRID No. 470176-24)

The objective of this study was to determine the potential for adverse effects of Vip3Aa19 insecticidal protein to freshwater fish using the channel catfish, *Ictalurus punctatus*, as a representative test species in a 30-day feeding study. The study compared survival and growth of juvenile channel catfish fed commercial fish feed formulated with transgenic corn grain with test substance FFPACHA-0100 (containing 7.1 µg Vip3Aa19 protein/g diet) or with non-transgenic corn grain for 30 days. Both feeds contained approximately 50% corn grain by weight. The diet was formulated using a "cold-pelleting" process to minimize exposure to temperatures that might degrade Vip3Aa19 insecticidal protein. The formulation, nutrient composition, characterization, homogeneity, and stability of the fish feed test substance was also analyzed. After 30 days, there was no test material-related mortality. Fish fed either the Vip3Aa19 corn grain or the control corn grain gained equal amounts of weight, and no abnormal behavior was observed in either group. The activity and stability of Vip3Aa19 in grain and fish feed was confirmed *via* fall armyworm insect bioassay and analyzed by enzyme-linked immunosorbent assay (ELISA) to confirm the presence and amount of the test material. There were no adverse effects on growth or behavior of juvenile catfish exposed for 30 days. Therefore, the NOEC was 7.1 µg Vip3Aa19/g diet and the 30-day LC_{50} was greater than 7.1 µg Vip3Aa19/g diet consisting of fish feed made from Event Pacha corn grain.

Conclusions/Recommendations: No observed adverse effects were noted in *Ictalurus punctatus* after exposure to Vip3Aa19 *via* commercial feed formulated from Event Pacha corn grain. The NOEC was 7.1 µg Vip3Aa19/g diet and the LC_{50} was greater than 7.1 µg Vip3Aa19/g diet consisting of fish feed made from Event Pacha corn grain. This study was previously reviewed and found acceptable (Waggoner and Vaituzis 2008a).

ii. Freshwater Aquatic Invertebrates (MRID No. 457921-01)

The objective of this study was to determine the potential for acute effects to the aquatic organism, *Daphnia magna*, during a static renewal exposure to Vip3Aa19 *via* pollen from Event Pacha corn. The test was conducted as a limit test using test substance PHOPACHA-0199, containing 83.8 µg Vip3Aa19 protein/g pollen. Daphnids were exposed to a single nominal test concentration of 120 mg pollen/L for 48 hours with renewal of the test solution at approximately 24 hours. Two control groups were included: a group in water exposed to pollen (120 mg/L) from non-transgenic, near-isogenic corn, and an assay control group exposed to water only. Each treatment was replicated three times and each replicate contained ten neonate daphnids. Observations of mortality, immobility and other sublethal effects were made during the test. At test termination, there was 100% survival in each group with no sign of immobilization or any other adverse effects. Therefore, the NOEC was 120 mg pollen/L and the LC_{50} was greater than 120 mg pollen/L.

Conclusions/Recommendations: Results of the 48-hour limit test showed the LC_{50} was greater than 120 mg pollen/L, representing 10.1 µg Vip3Aa19 protein/L. Based on the information presented, this study is unacceptable. The 48-hour test duration is not sufficient to show mortality for *Bt* toxins. The mode of action of the toxin would take more than 48 hours for target insect pests to succumb to *Bt* toxins; therefore, mortality or reproductive effects to aquatic invertebrates (e.g., daphnids) are not expected to show within 48 hours. Because Vip proteins are also derived from *Bt* and susceptible species display similar symptoms upon ingestion, a 7–14 day *Daphnia* study (OPPTS Guideline 885.4240) must be performed. This study 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 *Daphnia* study.

iii. Estuarine and Marine Animals – Waiver Granted

Estuarine and marine animal studies were not required for this product because of the low probability that estuarine or marine systems will be exposed to Vip3Aa20 insecticidal protein produced in Event MIR162 maize tissues and pollen.

Terrestrial and Aquatic Plant Species – Waiver Granted

Plant toxicity studies were not required for this product because Vip3Aa20 insecticidal protein, the active ingredient, is an insect toxin derived from *Bt* that has never shown any toxicity to plants.

Invertebrate Species

The Vip3Aa insecticidal protein is meant to target species within the order Lepidoptera (moths and butterflies). *Bacillus thuringiensis* toxins are known to have limited activity spectra across species; however, to address any unforeseen change in activity spectrum as a result of laboratory protein synthesis and to fulfill the published registration data requirements, EPA requires that test species used for non-target insect evaluations include several species that are not related to the target pests. Earthworm studies are also recommended on a case-by-case basis.

i. Ladybird Beetle (MRID Numbers 457665-09 and 468808-02)

MRID No. 457665-09
The purpose of this study was to determine the potential dietary effects of the Vip3Aa19 insecticidal protein on the mortality and development of the pink-spotted lady beetle, *Coleomegilla maculata*. The protocol for the non-target lady beetle study was based on OPPTS Guideline 885.4340. Eight- to nine-day-old lady beetles were exposed to Vip3Aa19 *via* Pacha corn pollen test substance (PHOPACHA-0100), which was incorporated into an artificial diet at 5% weight by weight (w/w). The negative control diet comprised 5% w/w pollen from non-transgenic, near-isogenic corn, and a positive control diet contained 50 µg thiobendacarb/g diet. The treatment and control groups each comprised three replicates of twenty-five beetles, which received fresh diet daily. After 21 days, there were no statistically significant differences in survival, development, and growth between the treatment and negative control groups ($P \leq 0.05$), while there was 100% mortality in the positive control group. Therefore, the NOEC was 7.24 µg Vip3Aa19/g diet and the LC_{50} was greater than 7.24 µg Vip3Aa19/g diet.

Conclusions/Recommendations: The results indicate that Vip3Aa19 insecticidal protein had no adverse effect on the survival, development, and growth of the lady beetles. The NOEC was 7.24 µg Vip3Aa19/g diet and the LC_{50} was greater than 7.24 µg Vip3Aa19/g diet. This study was previously reviewed and found acceptable (Rose and Vaituzis 2003).

MRID No. 468808-02
The objective of this study was to determine the potential dietary effects of Vip3Aa19 insecticidal protein on the mortality and development of the seven-spotted ladybird beetle, *Coccinella septempunctata.* The test substance, VIP3A-0204, was produced by recombinant *Escherichia coli* fermentation system and contained 7.25 mg Vip3Aa19/g before addition to a 50% sucrose diet. The negative control diet comprised of sucrose only, and a positive control diet contained 0.3333 mg dimethoate/g diet. Treatment and control groups, each comprising of 40 beetles, were fed fresh diet

daily and the endpoints of survival and development were evaluated through 15 days. At study end, mortality in the Vip3Aa19-treated group was not statistically significantly different from that of the untreated controls (0% vs. 5%, respectively). Positive control mortality was 100%. The NOEC was 7.25 mg Vip3Aa19 protein/g diet and the LC_{50} was greater than 7.25 mg Vip3Aa19 protein/g diet.

Conclusions/Recommendations: No adverse effects were seen in *C. septempunctata* after exposure to Vip3Aa19 insecticidal protein in a sucrose diet. The NOEC was 7.25 mg Vip3Aa19 protein/g diet and LC_{50} was greater than 7.25 mg Vip3Aa19 protein/g diet. This study was previously reviewed and found acceptable (Milofsky and Vaituzis 2007).

ii. Minute Pirate Bug (MRID No. 468848-14)

The purpose of this study was to determine the potential dietary effects of Vip3Aa19 insecticidal protein on mortality and development of *Orius insidiosus*, the minute pirate bug or insidious flower bug. The test substance was VIP3A-0104, a 63.1% pure preparation of microbially derived Vip3Aa19. The test substance was dissolved in buffer and incorporated at a rate of 11.49 mg/g diet (7.25 mg Vip3Aa19 protein/g of artificial diet – approximately 310x the highest mean concentration of Vip3Aa19 in COT102) and was continuously supplied to predatory bug (*Orius insidiosus*) nymphs for 21 days. Control nymphs were fed untreated diet, and positive control nymphs were fed diet treated with 10 µg teflubenzuron/g of diet. At study end, mortality in the Vip3Aa19-treated nymphs was not significantly different from that of the untreated controls (15% vs. 13%, respectively). Positive control mortality was 100%. The NOEC was 7.25 mg Vip3Aa19 protein/g diet and the LC_{50} value was greater than 7.25 mg Vip3Aa19 protein/g diet.

Conclusions/Recommendations: No adverse effects were seen in *Orius insidiosus* after exposure to Vip3Aa19 insecticidal protein in an artificial diet. The NOEC was 7.25 mg Vip3Aa19 protein/g diet and the LC_{50} value was greater than 7.25 mg Vip3Aa19 protein/g diet. This study was previously reviewed and found acceptable (Milofsky and Vaituzis 2007).

iii. Green Lacewing (MRID No. 468848-15)

The purpose of this study was to determine the potential dietary effects of Vip3Aa19 insecticidal protein on mortality and development of *Chrysoperla carnea* larvae, the green lacewing. The test substance, VIP3A-0104, consisted of 7.25 mg Vip3Aa19 protein/g of artificial diet and was continuously supplied to green lacewing (*Chrysoperla carnea*) larvae for 21 days. Control larvae were fed untreated diet, and positive control larvae were fed diet treated with 10 µg teflubenzuron/g diet. At study end, mortality in the Vip3Aa19-treated larvae was not statistically significantly different from that of the untreated controls (37.5% vs. 35.0%, respectively). Positive control mortality was 100%. Although the control mortality exceeded the 25% criterion for the test to be considered valid, mortality did not differ significantly between the test and control groups. Furthermore, the control mortality was <25% through day 21, which was judged to be a sufficient

exposure period to observe acute and developmental effects on lacewing larvae. Therefore, the NOEC was 7.25 mg Vip3Aa19 protein/g diet and the LC_{50} value was greater than 7.25 mg Vip3Aa19 protein/g diet.

Conclusions/Recommendations: No adverse effects were seen in *Chrysoperla carnea* after exposure to Vip3Aa19 insecticidal protein mixed in an artificial diet. The NOEC was 7.25 mg Vip3Aa19 protein/g diet and the LC_{50} value was greater than 7.25 mg Vip3Aa19 protein/g diet. This study was previously reviewed and found acceptable (Milofsky and Vaituzis 2007).

iv. Rove Beetle (MRID No. 471378-13)

The purpose of this study was to determine any reproductive effects of Vip3Aa20 insecticidal protein on *Aleochara bilineata* (rove beetle). In a laboratory bioassay, adult rove beetles (*Aleochara bilineata*) were exposed to a prepared meat diet containing 595.2 µg MIR162VIP3A-0106/g of diet for 35 days. The Vip3Aa20 concentration fed to the beetles was approximately 10 times that which occurs in fresh leaf tissue of Event MIR162 maize plants. A negative control diet and a reference control diet were also included in the test. To assess reproduction of the beetles, onion fly (*Delia antique*) pupae were provided to be parasitized by the beetles during the test. Second-generation beetles emerging from the parasitized pupae were counted until emergence stopped on test day 84. The results of the reproductive success of the beetles showed no statistically significant differences between the number of beetles that emerged from the Vip3Aa20 test treatment, when compared to the control. The International Organization for Biological Control (IOBC) validity criteria were met (Grimm *et al*. 2000) and the stability and bioactivity of the test material in the prepared diet were also confirmed. Therefore, no adverse effects were noted on the reproductive effects of Vip3Aa20 insecticidal protein on *A. bilineata*. Furthermore, the NOEC was 595.2 µg Vip3Aa20 protein/g diet for the reproduction of *A. bilineata* and the LC_{50} was greater than 595.2 µg Vip3Aa20 protein/g diet when exposed orally *via* a treated meat-based diet

Conclusions/Recommendations: No adverse effects were noted on the reproductive effects of Vip3Aa20 insecticidal protein on rove beetles. Therefore, the NOEC was 595.2 µg Vip3Aa20 protein/g diet for the reproduction of *A. bilineata* and the LC_{50} was greater than 595.2 µg Vip3Aa20 protein/g diet when exposed orally *via* a treated meat-based diet. Based on the results of the study, the data requirement for a representative, non-target coleopteran species is satisfied.

v. Collembola (MRID No. 458358-10)

The purpose of this study was to determine the potential dietary effects of Vip3Aa19 insecticidal protein on mortality and reproduction on *Folsomia candida* (springtail; Collembola). The test substances included LLPACHA-0100 (containing 43.4 µg Vip3Aa19 protein/g leaf tissue diet from Event Pacha corn), distilled water as a negative control, and thiodicarb as a positive control. There were four replicates of ten juvenile collembola per replicate per treatment and fresh diet was provided daily. Vip3Aa19 insecticidal protein had no detectable impact on the survival or

reproduction of the collembola after 28 days of continuous exposure. The NOEC of lyophilized Vip3Aa19 insecticidal protein from Event Pacha corn leaves was 50% of the diet, which was the highest concentration tested. Therefore, the NOEC was 43.4 µg Vip3Aa19 protein/g diet and the LC_{50} was greater than 43.4 µg Vip3Aa19 protein/g diet.

Conclusions/Recommendations: No adverse effects were seen on *Folsomia candida* after exposure to Vip3Aa19 protein in Event Pacha maize leaf tissue. The NOEC was 43.4 µg Vip3Aa19 protein/g diet and the LC_{50} was greater than 43.4 µg Vip3Aa19 protein/g diet. This study was previously reviewed and found acceptable (Rose and Vaituzis 2003).

vi. Honeybee (MRID No. 471479-01)

A semi-field, whole-hive feeding study was conducted based on the recommendations in European Plant Protection Organization (EPPO) Bulletin 22 (Oomen *et al*. 1992), and in accordance with the United Kingdom (UK) Good Laboratory Practice regulations of 1999 and Organization for Economic Cooperation and Development (OECD) principles [Revised 1997].

The objective of this study was to evaluate potential dietary effects of transgenic, microbially derived Vip3Aa20 on honeybee (*Apis mellifera*) larvae survival, adult emergence, exposed adult worker bee survival, and whole-hive conditions in a semi-field study. Honeybees were exposed, *via* oral ingestion, using in-hive commercial bee feeders. The treatments consisted of 71.4, 285.7, and 714.3 mg MIR162VIP3A-0106/L of sucrose solution, which corresponded to 50, 200, and 500 µg Vip3Aa20 protein/g of solution. The doses represent approximately 1x, 4x, and 10x the highest concentration of Vip3Aa20 in pollen of Event MIR162 maize. The test also included a negative control of 50% weight by volume (w/v) sucrose solution only and a reference control of 3 grams of diflubenzuron in 50% w/v sucrose solution. The test consisted of a single application of one liter of the appropriate solution per hive and the hives were observed for 24 days for percent successful brood development to adults and colony conditions. There was no significant difference in mortality between the test and negative control groups for brood development. There was also no significant difference in pre- and post-test hive conditions between the test and negative control treatments. Results for the positive control treatment were significantly different from the other treatments for brood development and hive condition (as indicated by the significantly reduced mean percentage of comb covered by life stages). Adult bees were not affected by any of the treatments. These results indicate direct and incidental ingestion of Vip3Aa20 insecticidal protein did not adversely affect brood development, exposed worker bees, or the hive condition. Therefore, the NOEL was 500 µg Vip3Aa20 protein/g of sucrose solution and the LD_{50} was greater than 500 µg Vip3Aa20 protein/g of sucrose solution.

Conclusions/Recommendations: No adverse effects were observed, after a single-dose application of MIR162VIP3A-0106 test material mixed with a sucrose solution, on *Apis mellifera* larvae, adult emergence, exposed adult worker bee survival, or whole-hive conditions after 24 days. The NOEL

was 500 µg Vip3Aa20 protein/g of sucrose solution and the LD_{50} was greater than 500 µg Vip3Aa20 protein/g of sucrose solution. Based on the results of the study, the data requirement for the honeybee toxicity is satisfied.

vii. Earthworm (MRID No. 457921-02)

The objective of this study was to evaluate the potential effects of Vip3Aa19 from Event Pacha corn administered to earthworms (*Eisenia fetida*) *via* an artificial soil substrate during a 14-day exposure period. The testing was conducted based on OPPTS Guideline 850.6200 (Earthworm Subchronic Toxicity Test) and OECD Guideline 207. In the test, earthworms were exposed to a single concentration of Vip3Aa19 insecticidal protein derived from Event Pacha corn leaves (test substance LPPACHA-0199) and incorporated into an artificial soil substrate at 3.60 mg Vip3Aa19 protein/kg soil. There were no mortalities in the assay control group, buffer control group, or Vip3Aa19 protein group. Analysis of the test soil showed that Vip3Aa19 was present in the soil and was biologically active against *Agrotis ipsilon* (black cutworm). Therefore, no adverse effects on earthworms were observed after exposure to Vip3Aa19 insecticidal protein *via* Event Pacha corn leaf tissue. The NOEC was 3.60 mg Vip3Aa19 protein/kg dry soil and the14-day LC_{50} for earthworms was determined to be greater than 3.60 mg Vip3Aa19 protein/kg dry soil.

Conclusions/Recommendations: No adverse effects from Vip3Aa19 corn leaf tissue in Event Pacha were seen on the survival of *Eisenia fetida via* an artificial soil substrate after 14 days. The NOEC was 3.60 mg Vip3Aa19 protein/kg dry soil and the14-day LC_{50} for earthworms was greater than 3.60 mg Vip3Aa19 protein/kg dry soil. This study was previously reviewed and found acceptable (Waggoner and Vaituzis 2008a).

b. Soil Fate

Soil organisms may be exposed to Vip3Aa insecticidal protein through contact with corn plant roots (by direct feeding), corn plant root exudates, incorporation of above-ground plant tissues into soil following harvest, or by soil-deposited pollen. Some evidence suggests that soils that are high in clays and humic acids are more likely to bind Cry protein. However, neutral-pH soils tend to have high microbial activity and microbes contribute to Cry protein degradation. The weight of evidence indicates that Cry proteins do not accumulate in soil to arthropod-toxic levels. Because Vip and Cry proteins are both toxins derived from soil-inhabiting bacteria, *Bacillus thuringiensis,* and found in commercial microbial insecticides (de Maagd *et al*. 2003; Graser and Song 2006), Vip protein degradation would also be similar to Cry protein degradation. The Agency previously reviewed the following soil fate evaluation to support Event Pacha corn expressing Vip3Aa19 insecticidal protein (Waggoner and Vaituzis 2008a). Because Vip3Aa19 and Vip3Aa20 are 99.9% identical, this study would also be applicable to the MIR162 *Bt* maize registration.

MRID No. 470176-30
The purpose of this study was to investigate the degradation of Vip3Aa19 insecticidal protein in various types of soils (clay, sandy clay loam, sandy loam, silt loam, and artificial soils) by assessing the loss of bioactivity *via* insect bioassay. The test substance, LPPACHA-0199 (corn leaf protein containing *ca*. 0.36% Vip3Aa19), was incorporated at concentrations of 16 or 4 mg Vip3Aa19 protein/g of soil and incubated under controlled conditions for 29 days. During the incubation, soil samples were collected weekly and used in black cutworm (BCW, *Agrotis ipsilon*) bioassays to determine biological activity of the test substance against the insect over time. The loss of bioactivity was measured by BCW mortality, which was used to estimate the DT_{50} (i.e., time to dissipation of 50% of the initial bioactivity) of the 16 mg Vip3Aa19 protein/g concentration of the test material in each soil. The estimated DT_{50} values ranged from 6.0 days in the silt loam to 12.6 days in one of the clays, indicating that Vip3Aa19 insecticidal protein in plant residues incorporated into soil is not likely to persist or accumulate in soil.

Conclusions/Recommendations: This study utilized field soil spiked with purified insecticidal protein derived from corn leaves. The reviewed data show that Vip proteins will be quickly degraded upon release from decaying plant tissue. This study was previously reviewed and found acceptable (Waggoner and Vaituzis 2008a).

Based on FIFRA Scientific Advisory Panel recommendations and public comments, the Agency has required a three-year soil fate study as a condition of registration for the currently registered Cry protein-producing *Bt* crops grown in a variety of soils and environmental conditions. The results of these studies show that there is no detectable Cry protein accumulation in agricultural soils during commercial planting of currently registered Cry protein-producing *Bt* crops (Milofsky and Vaituzis 2006).

More recently, a comprehensive review of all available scientific data on ecological effects of commercially grown GM crops over the last ten years was completed (Sanvido *et al*. 2007). The review concluded "none of the laboratory or field studies suggest accumulation of *Bt* toxins in soil over several years of cultivation" and "experience from commercial cultivation indicates that *Bt* toxin will not persist for long periods under natural conditions." The Agency agrees with these conclusions.

Collectively, the long-term field studies for *Bt* crops also confirm the previous SAP conclusion that "bioaccumulation is not expected to occur with transgenic proteins because *biodegradation mechanisms for proteins are ubiquitous*" (U.S. EPA 2000). More importantly, the numerous laboratory studies that demonstrated rapid protein degradation in soil of *Bt* proteins produced in *Bt* crops (when performed under realistic environmental conditions) can be considered predictive that *Bt* protein in soil is not likely to persist or accumulate in soil after continuous cultivation.

In light of these published findings and the rapid degradation of Vip3Aa19 insecticidal protein in soil as demonstrated in the insect bioassay, there is no indication that the Vip3Aa20 insecticidal protein expressed in Event MIR162 maize is likely to persist or accumulate in soil after continuous cultivation. Therefore, no additional long-term field studies are required for this PIP product.

c. Effects on Soil Microorganisms

Numerous published studies indicate that exposure to Cry protein produced in *Bt* PIP crop plants does not adversely affect soil microorganisms (Sanvido *et al*. 2007; Oliveira *et al*. 2008). In addition, *Bacillus thuringiensis* toxin released from root exudates and biomass of *Bt* corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil (Saxena and Stotzky 2001). Other research findings conclude no *Bt*-related risks have evolved from the decomposition of *Bt*-corn leaves for the meso- and macrofauna soil community (Hönemann *et al*. 2008). Although a minimal transient increase and shift in microbial populations may result from the presence of transgenic plant tissue in soil, no adverse effects have been attributed to the Cry protein. The Vip3Aa insecticidal protein had a similar DT_{50} or degradation time to Cry proteins and these proteins are both *Bt* toxins.

In addition, there are several ongoing U.S. Department of Agriculture and EPA Office of Research and Development funded research projects evaluating the effects of *Bt* crops on soil microbial flora. If adverse effects are seen from this or any other research, the Agency will take appropriate action to mitigate potential risks.

With regard to the impact of genetically engineered crops on soil, it is important to note that agricultural practices themselves cause large changes in soil and soil microbial composition. Furthermore, factors such as variations in seasons and weather, plant growth stage, and plant varieties, independent of being genetically engineered, are also responsible for significant shifts in soil microbial communities. To date, most studies with genetically engineered crops have shown minor or no effects on soil microbes beyond the variation caused by the factors listed above.

d. Horizontal Transfer of Transgenes from Bt Crops to Soil Organisms

The EPA has evaluated the potential for horizontal gene transfer (HGT) from *Bt* crops to soil organisms and has considered possible risk implications if such a transfer were to occur. Genes that have been engineered into *Bt* crops are mostly found in, or have their origin in, soil-inhabiting bacteria. Soil is also the habitat of anthrax, tetanus and botulinum toxin-producing bacteria. Transfer of these genes and/or toxins to other microorganisms or plants has not been detected. Furthermore, several experiments (published in scientific journals), that were conducted to assess the likelihood of HGT, have been unable to detect gene transfer under typical environmental conditions. Horizontal gene transfer to soil organisms has only been detected with very promiscuous microbes under laboratory conditions designed to favor transfer.

As a result of these findings, which suggest that HGT is at most an artificial event, and the fact that the *Bt* toxin engineered into MIR162 maize is derived from soil-inhabiting bacteria, the EPA has concluded that there is a low probability of risk from HGT of transgenes found in MIR162 maize expressing Vip3Aa20 insecticidal protein.

e. Gene Flow and Weediness Potential

Movement of transgenes from crop plants into weeds is a significant concern, due to uncertainty regarding the effect that a new pest resistance gene may have on plant populations in the wild. Under FIFRA, the EPA has reviewed the potential for gene capture and expression of *Bt* endotoxins by wild or weedy relatives of corn, cotton, and potatoes in the U.S., its possessions, or its territories. Because Vip proteins are *Bt* toxins and have similarities to Cry proteins in their insecticidal activity on similar target species, the Agency maintains the same approach in evaluation of gene flow and weediness potential. To date, *Bt* plant-incorporated protectants have been registered for use in agronomic plant species that do not have a reasonable possibility of passing their traits to wild, native plants. However, due to concern over the possibility that species related to corn (*Zea mays* ssp. *mays*), such as *Tripsacum* species and the teosintes, could be recipients of gene flow from genetically modified *Z. mays*, EPA conducted a thorough review of the scientific literature on what is known about the gene flow potential of *Z. mays* (U.S. EPA 2001b).

Conclusions gathered from this review process are as follows:

- The potential for pollen-directed gene flow from corn to Eastern gamagrass is extremely remote (DeWald *et al*. 1999b). This is evidenced by the difficulty with which *Tripsacum dactyloides* x *Z. mays* hybrids are produced in structured breeding programs. Additionally, the genus *Zea* does not represent any species considered as serious or pernicious weeds in the United States or its territories (Holm *et al*. 1979). Any introgression of genes into this species as a result of cross fertilization with genetically modified corn is not expected to result in a species that is weedy or difficult to control. In many instances where hybridization has been directed between these two species, the resultant genome is lacking in most or all of the corn chromosomal complement in subsequent generations (DeWald, personal communication, 1999a).

- Many of the *Zea* species loosely referred to as "teosintes" will produce viable offspring when crossed with *Zea mays* ssp. *mays*. However, none of these plants are known to harbor weedy characteristics and none of the native teosinte species, subspecies, or races are considered to be aggressive weeds in their native or introduced habitats (Schoper, personal communication, 1999). In fact, many are on the brink of extinction where they are indigenous and will be lost without human intervention (i.e., conservation measures). Further, none of the landraces or cultivated lines of *Z. mays* are considered to have weedy potential and are generally

considered to be incapable of survival in the wild as a result of breeding practices (i.e., selection) during domestication of the crop.

The October 2000 Scientific Advisory Panel agreed that the potential for gene transfer between corn and any receptive plants within the U.S., its possessions, or its territories was of limited probability and nearly risk free. Based on these findings, the EPA has determined that there is no significant risk of gene capture and expression of Vip3Aa20 protein by wild or weedy relatives of corn in the U.S., its possessions, or its territories.

f. Impacts on Endangered Species

The primary route of exposure to Vip3Aa20 protein in maize is through ingestion of maize tissue. There are no reports of threatened or endangered species feeding on maize plants; therefore, such species would not be exposed to maize tissue derived from Event MIR162. In addition, Vip3Aa insecticidal protein has shown no toxic effects on mammals, birds, plants, aquatic species, insects, and other invertebrate species at the Estimated Environmental Concentration (EEC); therefore, a "may affect" is not anticipated for endangered land and aquatic species. Moreover, EPA does not expect that any threatened or endangered plant species will be affected by outcrossing to wild relatives or by competition with such entities. Hybrid maize does not exist in the wild, nor are there wild plants that can interbreed with maize in the United States.

Because of the selectivity of Vip3Aa proteins for lepidopteran species, endangered species concerns are mainly restricted to the order Lepidoptera. Examination of an overlay map showing the county-level distribution of endangered/threatened lepidopteran species (currently listed by the U.S. Fish and Wildlife Service) relative to maize production counties in the United States clearly indicated that any potential concern regarding range overlap with maize production was mainly restricted to the Karner blue butterfly (*Lycaeides melissa samuelis*). Research demonstrates that Vip3Aa proteins are selectively toxic to lepidopteran larvae at field concentrations and that the Karner Blue butterfly is the only endangered lepidopteran species that may be exposed to MIR162 maize (*via* pollen).

After careful review of available data, EPA determined that exposure of the Karner blue butterfly to harmful levels of MIR162 maize plant tissue is not expected. Likewise, a review of the preferred habitats of other lepidopteran species listed as endangered by the U.S. Fish and Wildlife Service indicated that exposure to harmful levels of Vip3Aa20 insecticidal protein would not take place. The main reasons for the lack of exposure are geographical and habitat limitations. These species are located in non-maize production areas and/or their habitat does not encompass agricultural areas.

Likewise, other insect species in the orders Diptera, Hemiptera, Lepidoptera, Odonata and Orthoptera that are listed as endangered/threatened species are found in dune, meadow/prairie, or open forest habitats and are not closely associated with row crop production, often times due to the specificity of the habitat of their host plants. The reviewed toxicological data shows the relative insensitivity of a range of insects in non-lepidopteran orders to the Vip3Aa20 insecticidal protein,

indicating that MIR162 maize is not likely to have detrimental effects on non-lepidopteran insects included on the endangered/threatened species list.

In light of the above considerations (based on no spatial and temporal overlap), the Agency has determined that registered uses of MIR162 maize will have No Effect (NE), direct or indirect, on endangered and threatened species or their habitat as listed by the United States Fish and Wildlife Service (USFWS) and the National Marine Fisheries Services (NMFS), including mammals, birds, terrestrial and aquatic plants, and invertebrate species. Therefore, no consultation with the USFWS is required under the Endangered Species Act.

iv. Environmental Risk Assessment for Event MIR162 Maize

The EPA uses a maximum hazard dose tiered system for the non-target wildlife hazard assessment for biopesticides. When no adverse effects at the maximum hazard screening dose are observed, the Agency concludes that there are no unreasonable adverse effects from the use of the pesticide.

a. *Direct Effects*

At present, 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 that 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 (see section II(C)(1)(iii)(f)). 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 (see section II(C)(1)(iii)(e)). Available data do not indicate that Cry or Vip proteins have any measurable adverse effect on microbial populations in the soil (see section II(C)(1)(iii)(c)), nor has any horizontal transfer of genes from transgenic plants to soil bacteria been demonstrated (see section II(C)(1)(iii)(d)). In conclusion, this risk assessment finds no hazard to the environment at the present time from cultivation of Event MIR162 maize expressing Vip3Aa insecticidal protein.

b. Indirect Effects

The purpose of using PIP plants is the same as for any other pest management tactic, i.e., to reduce pest populations below economic injury levels. As a result, the abundance of pest insects should be significantly reduced and this will have corresponding implications for those organisms that exploit

these pests as prey and hosts. Thus, the potential for these indirect ecological effects on biological control organisms should not be regarded as a unique ecological risk associated with the PIP crop. Some reductions, however, should be expected if the pest management strategy is effective. Since PIP crops are often grown in the vicinity of conventional crops to prevent resistance build up by the target pest(s), specialist antagonists can persist in these "refuges," in other crops, and in non-crop habitats and retain the potential for recolonization of the PIP crop area. Based on these considerations, regulatory testing of the specialist predators and parasitoids of target pests may eventually be considered unnecessary.

2. Supplemental Data Needed to Confirm MIR162 Non-Target Hazard Assessment

The Agency has sufficient information to believe that there is no risk from the proposed uses of Event MIR162 maize to non-target wildlife, aquatic, and soil organisms. In previous Section 3 registrations of PIPs, the Agency required registrants to conduct post-registration, long-term invertebrate population/community studies and protein accumulation in soils studies. However, the issue of long-range effects of cultivation of these Cry proteins on the invertebrate community structure in maize and cotton fields has since been adequately addressed by the meta-analysis of field studies performed during the last 10 years (Marvier *et al*. 2007; Sanvido *et al*. 2007). No unexpected adverse effects on invertebrate community structure were reported (Dively *et al*. 2005). The Agency is in agreement with these conclusions. Likewise, no unexpected accumulation of Cry proteins in agricultural soils was seen in published studies (Icoz and Stotzky 2007; Sanvido *et al*. 2007) and in numerous studies submitted directly to the EPA for the currently registered *Bt* PIP products containing Cry or Vip proteins (Milofsky and Vaituzis 2006; Waggoner and Vaituzis 2008a; see section II(C)(1)(iii)(b)).

However, in light of the published laboratory studies showing reduced growth in shredding caddis flies exposed to anti-lepidopteran Cry1A protein maize litter (Rosi-Marshall *et al*. 2007), additional aquatic invertebrate data are required. The submitted *Daphnia magna* study is unacceptable because it is an 850 Series OPPTS Guideline study. The 48-hour duration of this study is not sufficient to detect mortality. It takes more than 48 hours for the target pests to succumb to *Bt* toxins, such as Cry or Vip proteins; therefore, 48 hours is also not expected to show mortality or reproductive effects on *Daphnia*. A 7–14 day *Daphnia* study as per the OPPTS Guideline 885.4240 must be performed (see Table 2) for Event MIR162 maize. 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 *Daphnia* study. This study must be submitted as a condition of registration.

Table 2. Supplemental Non-Target Data Requirements for MIR162 Expressing Vip3Aa20

Testing Category	Type of Data
Aquatic Invertebrate	A 7−14 day *Daphnia* study as per the OPPTS Guideline 885.4240 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 *Daphnia* study.

3. Environmental Risk Assessment for *Bt*11 x MIR162 and *Bt*11 x MIR162 x MIR604 Corn Hybrids

This is an addendum to the Agency's environmental risk assessment for Vip3Aa20 insecticidal protein expressed in Event MIR162 maize reviewing MIR162's associated combined-PIP products, *Bt*11 x MIR162 and *Bt*11 x MIR162 x MIR604 corn (Waggoner and Vaituzis 2008b). The MIR162 environmental risk findings are described in sections II(C)(1)–(2) of this chapter.

The second protein expressed in the *Bt*11 x MIR162 combined-PIP product is Cry1Ab insecticidal protein, providing protection against the European corn borer and other lepidopteran pests. The Cry1Ab insecticidal protein produced in Event *Bt*11 corn (EPA Registration [Reg.] No. 67979-1 for field corn; EPA Reg. No. 65268-1 for sweet corn) was reassessed in 2001 (U.S. EPA 2001b) and the *Bt*11 environmental risk findings are summarized in section II(C)(3)(ii) of this chapter.

The third protein expressed in the *Bt*11 x MIR162 x MIR604 combined-PIP product is modified Cry3A (mCry3A), which provides resistance to western corn rootworm and northern corn rootworm. The mCry3A protein produced in Event MIR604 corn (EPA Reg. No. 67979-5) and its associated stacked product, *Bt*11 x MIR604 corn (EPA Reg. No. 67979-8), were granted Section 3 registrations in 2006 and 2007, respectively (U.S. EPA 2007). The MIR604 environmental risk findings are summarized in section II(C)(3)(iii) of this chapter.

i. Event MIR162 (Lepidopteran Active) Environmental Risk Assessment

Potential adverse effects to non-target organisms by Vip3Aa insecticidal protein have been reviewed (Waggoner and Vaituzis 2008a, b). A summary of the Vip3Aa environmental risk assessment from Waggoner and Vaituzis (2008b) is described in sections II(C)(1)–(2) of this chapter.

ii. Event *Bt*11 (Lepidopteran Active) Environmental Risk Assessment

Potential adverse effects to non-target organisms by Cry1Ab insecticidal protein have been reviewed (U.S. EPA 2001b). The following is a summary of the Cry1Ab environmental risk assessment.

Prior to registration of the first *Bt* plant-incorporated protectants in 1995, EPA conducted ecological risk assessments for all *Bt* Cry proteins expressed in potato, corn, and cotton. EPA evaluated studies of potential effects on a wide variety of non-target organisms that might be exposed to the *Bt* protein (U.S. EPA 2001b). This included Cry1Ab insecticidal protein as expressed in Syngenta's Event *Bt*11 corn. EPA performed risk assessments on plants, wild mammals, birds, fish, aquatic invertebrates, estuarine and marine animals, earthworms, terrestrial non-target insects (including honey bee adults and larvae, parasitic wasps, green lacewings, several lady beetle species, springtails [collembola toxicity/reproduction], and monarch butterflies), field evaluations of the effects of Cry1Ab exposure on non-target invertebrates, soil degradation/persistence studies, and endangered species impacts (U.S. EPA 2001b). In addition, gene flow and weediness assessments *via* pollen and Cry protein DNA uptake by plants and soil microorganisms were also performed. EPA concluded that there is sufficient information to believe that there is no risk to non-target wildlife, aquatic organisms, and soil organisms from the uses of Cry1Ab corn.

At present, the Agency is aware of no identified significant adverse effects of Cry protein on the abundance of non-target organisms in any population in the aquatic or terrestrial field environment, whether they are animals, plants, pest parasites, pest predators, or pollinators. Field testing and field census data submitted to the Agency show minimal to undetectable changes in beneficial insect abundance or diversity. In corn fields, densities of predatory and non-target insects are generally higher on Cry1Ab corn than non-*Bt* corn. Multi-year invertebrate abundance studies do not show a shift in biodiversity in Cry1Ab corn fields, except in cases where the predators are dependent on the pest insect as prey. In contrast, treatment with chemical pesticides, when studied, had significant effects on the total numbers of insects and on the numbers within the specific groups. To date, the available field test data show that transgenic crops have no detrimental effect on the abundance of non-target invertebrate populations when compared to crops treated with conventional chemical pesticides.

The movement of Cry1Ab transgenes from the host plant into weeds and other crops was also considered. The Agency determined that there is no significant risk of gene capture and expression of Cry1Ab insecticidal protein by wild or weedy relatives of corn in the U.S., its possessions, or its territories. The fate of Cry1Ab insecticidal protein in soils and indirect effects on soil biota have also been evaluated (U.S. EPA 2001b). The data show that most of the Cry protein deposited into soil is quickly degraded, although a residual amount may persist in biologically active form for a much longer period of time (Milofsky and Vaituzis 2006). It is also reported that the same degree of *Bt* Cry protein persistence takes place in soils that have been exposed to repeat *Bt* spray applications when compared to soil exposed to growing *Bt* crops. Limited data do not indicate that

Cry proteins have any measurable effect on microbial populations in the soil. Horizontal transfer from transgenic plants to soil microbes has not been demonstrated (Sanvido *et al*. 2007). Published studies looking at *Bt* Cry proteins in soil show no effect on bacteria, actinomyces, fungi, protozoa, algae, nematodes, springtails, or earthworms (Saxena and Stotzky 2001). In addition, new plants grown in *Bt* Cry protein-containing soil do not take up the *Bt* protein.

In conclusion, the risk assessment found no hazard to the environment from cultivation of Event *Bt*11 corn expressing Cry1Ab insecticidal protein.

iii. Event MIR604 (Coleopteran Active) Environmental Risk Assessment

Potential adverse effects to non-target organisms by mCry3A insecticidal protein has been reviewed (U.S. EPA 2007). The following is a summary of the mCry3A environmental risk assessment.

For registration of mCry3A as expressed in Event MIR604 corn, EPA reviewed studies conducted on representative non-target species and performed risk assessments on plants, wild mammals, birds, fish, aquatic invertebrates, estuarine and marine animals, earthworms, terrestrial non-target insects (including honey bee adults and larvae, rove beetles, minute pirate bugs, carabid beetles, and lady beetles), and soil degradation/persistence studies (U.S. EPA 2007). In addition, gene flow and weediness assessments *via* pollen and Cry protein DNA uptake by plants and soil microorganisms were also performed. EPA concluded that there is sufficient information to believe that there is no risk to non-target wildlife, aquatic organisms, and soil organisms from the uses of mCry3A corn.

An endangered species impact assessment of possible effects on Hungerford's crawling water beetle was performed for this registration. Hungerford's crawling water beetle is currently known to occur in only six streams—five in mostly northern Michigan and one in Ontario, Canada. These are not major corn-growing areas. The beetles are found in the cool riffles of clean, slightly alkaline streams. All streams where this beetle has been found have moderate to fast water flow, good stream aeration, and inorganic substrate. Often, these streams also have an open to partially open canopy just below beaver dams or similar human-made structures. Adults prefer gravel and cobble riffles while larvae occupy areas with slower current and dense growth of microalgae, especially Chara. Since the Hungerford's crawling water beetle larvae are reported to feed on filamentous algae (and possibly periphytic diatoms), no dietary exposure to anti-coleopteran Cry protein in corn tissue is expected. Therefore, the No Effect (NE) finding, direct or indirect, from cultivation of anti-coleopteran Cry protein containing corn to Hungerford's crawling water beetle was confirmed.

At present, the Agency is aware of no identified significant adverse effects of Cry protein on the abundance of non-target organisms in any population in the aquatic or terrestrial field environment, whether they are animals, plants, pest parasites, pest predators, or pollinators. Field testing and field

census data submitted to the Agency show minimal to undetectable changes in beneficial insect abundance or diversity. In corn fields, densities of predatory and non-target insects are generally higher on mCry3A corn than non-*Bt* corn. Two-year invertebrate abundance studies do not show a shift in biodiversity in mCry3A corn fields, except in cases where the predators are dependent on the pest insect as prey. In contrast, treatment with chemical pesticides, when studied, had significant effects on the total numbers of insects and on the numbers within the specific groups. To date, the available field test data show that transgenic crops have no detrimental effect on the abundance of non-target invertebrate populations when compared to crops treated with conventional chemical pesticides.

Furthermore, the EPA has determined that there is no significant risk of gene capture and expression of mCry3A insecticidal protein by wild or weedy relatives of corn in the U.S., its possessions, or its territories. The fate of mCry3A protein in soils and indirect effects on soil biota have also been evaluated (U.S. EPA 2007). Available data do not indicate that Cry proteins have any measurable adverse effect on microbial populations in the soil, nor has horizontal transfer of genes from transgenic plants to soil microbes been demonstrated.

In conclusion, the risk assessment found no hazard to the environment from cultivation of Event MIR604 corn expressing mCry3A insecticidal protein.

iv. Synergism Studies

The purpose of these studies was to characterize the potential for interaction between the lepidopteran-active proteins (Vip3Aa and Cry1Ab) and the coleopteran-active protein (mCry3A). In order to bridge the ecological effects and environmental fate data of the individual parental events to the combined-PIP products, the effects of the pesticidal mixture of the combined-PIP product must be tested on a susceptible pest species *via* diet-incorporation bioassays. Interactions between the test materials can be assessed by comparing the larval mortality observed for the mixed proteins with the predicted responses based on the bioassay of each protein individually. If there is no greater mortality than expected over the range of concentrations in a sensitive pest species, it is likely that there will be no synergism of the mixture against non-target organisms.

a. Potential Interactions in Between Cry1Ab and mCry3A Proteins

MRID No. 467956-04

A study was conducted to assess the combined effects of Cry1Ab and mCry3A insecticidal proteins on two sensitive insect species: European corn borer (ECB, *Ostrinia nubilalis*) and Colorado potato beetle (CPB, *Leptinotarsa decemlineata*). A series of dilutions were conducted in which first-instar ECB and CPB larvae were exposed to a high and a low concentration of the first protein (Cry1Ab or mCry3A), represented by the LC_{70} and LC_{30}, respectively, in combination with a high concentration

of the second protein (mCry3A or Cry1Ab), represented by the LC_{90} to the corresponding sensitive species. Neither the ECB nor the CPB data revealed evidence of synergistic or antagonistic interactions between Cry1Ab and mCry3A after analysis of the proportional mortality of sensitive bioassay species at the intended endpoint of the experiments. These results indicated that the effect of a mixture of mCry3A and Cry1Ab on non-target Lepidoptera and Coleoptera species can be predicted from the effects of the individual proteins alone (Hunter and Vaituzis 2007; U.S. EPA 2007).

b. Potential Interactions in Between Vip3Aa and Cry1Ab Proteins

MRID No. 470176-21

Four laboratory feeding bioassays were conducted to assess any synergistic or antagonistic interactions between Vip3Aa and full-length Cry1Ab (FLCry1Ab) insecticidal proteins in the lepidopteran pest, tobacco budworm (TBW, *Heliothis virescens*). Five dilution series of the test materials were prepared in buffer for each test: one series each of Vip3Aa and FLCry1Ab alone, and three series of the two proteins mixed together in different ratios (up to 1,600 µg/mL Vip3Aa and 100 µg/mL FLCry1Ab together). There was no evidence of either a synergistic or an antagonistic interaction between Vip3Aa and FLCry1Ab in *H. virescens*, indicating that the effect of a mixture of Vip3Aa and FLCry1Ab on non-target Lepidoptera can be predicted from the effects of the individual proteins alone.

MRID No. 470176-22

Three laboratory feeding bioassays were conducted to assess any synergistic or antagonistic interactions between Vip3Aa and full-length Cry1Ab insecticidal proteins in the lepidopteran pest, cotton bollworm (CBW, *Helicoverpa zea*). Five dilution series of the test materials were prepared in buffer for each test: one series each of Vip3Aa and FLCry1Ab alone, and three series of the two proteins mixed in different ratios (up to 25,600 nanograms [ng]/square centimeter [cm^2] Vip3Aa and 12,800 ng/cm^2 FLCry1Ab together). No evidence of either a synergistic or an antagonistic interaction between Vip3Aa and FLCry1Ab was observed in *H. zea*, indicating that the effect of a mixture of Vip3Aa and FLCry1Ab on non-target Lepidoptera can be predicted from the effects of the individual proteins alone.

Conclusions/Recommendations: The results of the interaction studies from subsets of the combined proteins (Cry1Ab and mCry3A against ECB and CPB; and Vip3Aa and FLCry1Ab against TBW and CBW) indicate that there is no change in the level of activity among susceptible insects. Collectively, these data provide evidence that Vip3Aa, Cry1Ab, and mCry3A insecticidal proteins do not interact in an antagonistic or synergistic manner. These data were previously reviewed and found acceptable by the Agency (Hunter and Vaituzis 2007; Waggoner and Vaituzis 2008a).

v. Effects of Combined-PIP Products on Non-Target Organisms

The potential for interaction among the Cry1Ab, Vip3Aa20, and mCry3A insecticidal proteins was tested using three species of non-target organisms: the rove beetle (*Aleochara bilineata*) and the pink-spotted lady beetle (*Coleomegilla maculata*), which are related to the target pest of mCry3A in MIR604 corn; and the monarch butterfly (*Danaus plexippus*), which is sensitive to Cry1Ab.

a. Rove Beetle (MRID No. 471530-05)

Adult rove beetles were exposed to a mixture of 50 µg Vip3Aa20 + 15 µg Cry1Ab + 25 µg mCry3A per gram of a meat-based diet for 35 days. The microbially produced protein concentrations were chosen to represent at least the highest concentrations in the tissues of corn plants derived from the relevant events, or in breeding stacks containing the transgenes introduced in these events. Reproduction of beetles fed the diet containing the test materials was compared with that of control beetles fed untreated diet or diet containing the buffer used to dissolve Vip3Aa20 and mCry3A in the test material diet. There was no statistically significant difference in reproduction of the test material group compared to the control groups. Previous studies also found no effect of mCry3A and Vip3Aa19 on the rove beetle (U.S. EPA 2007, 2008), and Cry1Ab is not known to be toxic to Coleoptera at the concentrations found in *Bt*11 corn.

Conclusions/Recommendations: No adverse effects were seen on the reproduction of rove beetles after exposure to the combined effects of Vip3Aa20, Cry1Ab, and mCry3A insecticidal proteins in a treated, meat-based diet. The NOEC was greater than 50 µg Vip3Aa20 + 15 µg Cry1Ab + 25 µg mCry3A per gram of diet for the reproduction of *Aleochara bilineata* and the LC_{50} was greater than 50 µg Vip3Aa20 + 15 µg Cry1Ab + 25 µg mCry3A per gram of diet, when exposed orally *via* a treated, meat-based diet.

b. Ladybird Beetle (MRID No. 471372-08)

Ladybird beetle (*Coleomegilla maculata*) larvae were exposed to 50 µg Vip3Aa20 + 11.23 µg Cry1Ab + 24 µg mCry3A per gram of a moth egg/bee pollen diet. The protein concentrations were chosen to represent at least the highest concentrations that would be present in tissues of corn hybrids derived from the relevant events, or breeding-stack hybrids derived from these events. There were no statistically significant differences in days to pupation or adulthood, pupal mortality, or percent larval or adult mortality for larvae fed the test material diet compared to larvae fed untreated control diet. Previous studies have shown no effects of Cry1Ab, mCry3A, and Vip3Aa19 on ladybird beetles (U.S. EPA 2007, 2008).

Conclusions/Recommendations: No adverse effects were seen in *C. maculata* after exposure to the combined effects of Vip3Aa20, Cry1Ab, and mCry3A insecticidal proteins in a moth egg/bee pollen diet. The NOEC was greater than 50 µg Vip3Aa20 + 11.23 µg Cry1Ab + 24 µg mCry3A per gram of diet on the development and survival of *Coleomegilla maculata* and the LC_{50} was greater than 50 µg

Vip3Aa20 + 11.23 µg Cry1Ab + 24 µg mCry3A per gram of diet, when exposed orally *via* a treated bee pollen and moth egg-based diet.

c. Monarch Butterfly (MRID No. 471372-10)

First-instar monarch butterfly larvae were exposed to non-transgenic corn pollen, *Bt*11 corn pollen, or *Bt*11 x MIR162 x MIR604 corn pollen at a density of 680 grains/cm^2 on leaves of the food plant, milkweed (*Ascelpias curassavica*). The larvae were fed pollen-treated leaves for four days, and then fed untreated leaves. In 2 separate runs of the test, the control mortality validity criterion of 20% was exceeded on day 6 and day 7, respectively. As a result, the data from day 5 and day 6 were analyzed for differences in mortality between the test material groups and control groups fed untreated leaves only. There was no significant difference in mortality of the test material pollen group compared to that of the control group in either run of the experiment.

Conclusions/Recommendations: No statistically significant differences were noted in mortality from the combined effects of *Bt*11 x MIR162 x MIR604 corn when compared to the assay control group on monarch butterfly larvae. In addition, no adverse effects were test treatment related and were attributed to experimental shortcomings of high control mortality. The NOEC was greater than 0.425 mg *Bt*11 x MIR162 x MIR604 corn pollen/cm^2 of milkweed leaf diet on *Danaus plexippus* larvae and the LC_{50} was greater than 0.425 mg *Bt*11 x MIR162 x MIR604 corn pollen/cm^2 diet, when exposed orally *via* a liquid-suspended test pollen and milkweed leaf diet.

vi. Field Studies

a. Efficacy Studies

MRID No. 476049-01

The efficacy of *Bt*11 corn, MIR162 maize, MIR604 corn, *Bt*11 x MIR162 corn, and *Bt*11 x MIR162 x MIR604 corn was compared against several pests (including black cutworm, fall armyworm, European corn borer, and western corn rootworm). The results were provided to the Agency as supplemental data to demonstrate the lack of interaction (i.e., no synergism or antagonism) among the insecticidal proteins produced in *Bt*11 corn, MIR162 maize, and MIR604 corn (MRID No. 476049-01; Waggoner and Kough 2009). The efficacy of *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn was consistent with an additive effect of the individual efficacies of *Bt*11 corn, MIR162 maize, and MIR604 corn alone in all the field studies (described in Huber *et al*. 2007; White *et al*. 2007a, b, c, and d). Therefore, the efficacy studies also support the lack of synergistic effects by showing no interaction between the Cry1Ab, Vip3Aa20, and mCry3A insecticidal proteins produced in the combined-PIP products events, *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn.

b. Non-Target Organism Field Studies

Another Vip3Aa protein variant is also expressed in the recently registered Event COT102 cotton as Vip3Aa19 and in Syngenta's experimental Event Pacha corn. Event Pacha corn also expresses Vip3Aa19 protein, which is over 99.9% identical to Vip3Aa20 protein (Barsoum and Kough 2008). In a three-year field study of *Bt*11 x Pacha corn, no significant differences in the composition of non-target organism communities were seen between *Bt*11 x Pacha corn and a non-transgenic, near-isogenic corn that was not treated with insecticide (Dively 2005).

vii. Overall Synergism Conclusion

The synergism studies, non-target organism toxicity testing, and field studies reviewed for the *Bt*11, MIR162, and MIR604 parental events indicate their associated combined-PIP products, *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn, will not result in any unexpected interaction related to an antagonistic or synergistic action to target and non-target insects. Therefore, it is extremely unlikely that the Vip3Aa, Cry1Ab, and mCry3A insecticidal proteins contained in a single plant will impart any hazard to non-target organisms exposed to these hybrids in the environment. The compilation of ecotoxicity studies on non-target organisms, evaluation for synergism between the test proteins, efficacy data, and field data support the bridging of the environmental risk assessment from the parental events to the combined-PIP *Bt*11 x MIR162 and *Bt*11 x MIR162 x MIR604 corn products.

viii. Supplemental Data Needed to Confirm *Bt*11 x MIR162 Corn and *Bt*11 x MIR162 x MIR604 Corn Non-Target Hazard Assessment

The Agency has sufficient information to believe that there is no risk from the proposed uses of *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn to non-target wildlife, aquatic, and soil organisms. In previous Section 3 registrations of PIPs, the Agency required registrants to conduct post-registration long-term invertebrate population/community studies and protein accumulation in soils studies. However, the issue of long-range effects of cultivation of crops containing these Cry proteins on the invertebrate community structure in corn and cotton fields has since been adequately addressed by the meta-analysis of field studies performed during 10 years (Marvier *et al*. 2007; Sanvido *et al*. 2007). No unexpected adverse effects on invertebrate community structure were reported (Dively 2005). The Agency is in agreement with these conclusions. Likewise, no unexpected accumulation of Cry proteins in agricultural soils was seen in published studies (Icoz and Stotzky 2007; Sanvido *et al*. 2007) and in numerous studies submitted directly to the EPA for the currently registered *Bt* PIP products containing Cry or Vip proteins (Milofsky and Vaituzis 2006; Waggoner and Vaituzis 2008a, b).

However, additional aquatic invertebrate data are required for the Event MIR162 maize product as a condition of registration, in light of the published laboratory studies showing reduced growth in shredding caddisflies exposed to anti-lepidopteran Cry1A protein maize litter (Rosi-Marshall *et al*.

2007). Therefore, a condition of registration for the MIR162 stacked and/or pyramided products is based on the registrant's data submission to satisfy the conditions of registration for the MIR162 parental event maize product. Furthermore, the Agency must find this data submission to be acceptable.

ix. Conclusion

Based on prior assessments conducted on the Vip3Aa, Cry1Ab, and mCry3A insecticidal proteins individually, the environmental risk assessment for the *Bt*11 x MIR162 and *Bt*11 x MIR162 x MIR604 corn hybrids indicates that no unreasonable harm will result to the environment or any Federally listed endangered or threatened species from commercial cultivation of these corn hybrids. The Agency has determined that the *Bt*11 x MIR162 and *Bt*11 x MIR162 x MIR604 corn hybrids will have No Effect (NE) on endangered and/or threatened species listed by the U.S. Fish and Wildlife Service (USFWS) and the National Marine Fisheries Services (NMFS), including mammals, birds, terrestrial and aquatic plants, and invertebrate species. Therefore, no consultation with the USFWS is required under the Endangered Species Act.

The Agency believes that cultivation of *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn may result in fewer adverse impacts to non-target organisms than result from the use of chemical pesticides. Under normal circumstances, *Bt* corn requires substantially fewer applications of chemical pesticides. This should result in fewer adverse impacts to non-target organisms because application of nonspecific conventional chemical pesticides is known to have an adverse effect on non-target beneficial organisms found living in the complex environment of an agricultural field. Many of these beneficial organisms are important integrated pest management (IPM) controls for secondary pests such as aphids and leafhoppers. Therefore, the overall result of cultivation of *Bt*11 x MIR162 corn expressing Cry1Ab and Vip3Aa20 insecticidal proteins, and *Bt*11 x MIR162 x MIR604 corn expressing Cry1Ab, Vip3Aa20, and mCry3A insecticidal proteins, is that the number of chemical insecticide applications for non-target pest control will be reduced for management of multiple pest problems.

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D. Insect Resistance Management (IRM)

1. Background

Syngenta's *Bt*11 x MIR162 x MIR604 corn is a stacked and pyramided transgenic corn product that expresses the two registered crystal protein toxins, Cry1Ab and mCry3A, and incorporates the novel *Bacillus thuringiensis* (*Bt*) Vip3Aa20 toxin, which is 99.9% identical in amino acid sequence to the Vip3Aa19 toxin produced in COT102 cotton (which is a component of a registered Cry1Ab x Vip3Aa19 cotton product). Vip3A is different from Cry proteins as it is produced during vegetative growth of the bacteria, does not form parasporal crystal proteins, and is secreted (but not processed upon secretion) from the cell as a soluble protein. While its physical manifestations of intoxication in sensitive larvae resemble those of Cry proteins (gut paralysis and lysis of midgut epithelial cells) (Schnepf *et al*. 1998), activated Vip3A does not bind to the same receptors (aminopeptidase-N [APN] and cadherin-like receptors, in the case of Cry1Ab). These two types of *Bt* proteins (Vip3Aa, Cry1Ab) do not appear to share binding sites. Lee *et al*. (2003) have investigated the mode of action of the Vip3A protein and determined that it involves a number of steps much like the modes of action for the delta-endotoxins. Following ingestion by the lepidopteran target pest, Vip3A protein becomes soluble in the gut and is processed into four dominant bands (retaining activity). The authors propose that this processing is required for the bioactivity of the toxin (activation step). Interaction with the midgut epithelium is the next likely step in the mode of action of Vip3A. However, Vip3A does not bind to APN and cadherin-like glycoprotein receptors. Upon binding to midgut epithelial receptors, data support the existence of a pore-forming step that creates ion channels, which are structurally and functionally distinct from those of Cry1Ab. Direct structural information is missing for Vip3A; however, preliminary data do not support the notion that the two proteins share similar domain organization or an alpha-helical bundle region.

In 2006, the Agency reviewed Syngenta's IRM plan for the stacked product, *Bt*11 x MIR604 maize (Milofsky and Matten 2006). Based on efficacy and protein expression studies, the Agency decided that the IRM programs developed for the individual trait products should also be appropriate for the stacked product (i.e., 20% structured non-*Bt* refuge in corn-growing areas and 50% structured non-*Bt* refuge in cotton-growing areas), and in the case of a combined refuge strategy for lepidopteran and coleopteran pests, some modifications should apply (Matten 2006).

Syngenta received an experimental use permit (EUP) to allow field testing of the plant-incorporated protectant (PIP), Event MIR162 maize, and its combined-trait varieties, *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn, in 23 states to cover the period from March 1, 2007 through February 29, 2008. Event MIR162 maize expresses the Vip3Aa20 insect control protein. Vip3Aa20 has insecticidal activity against several lepidopteran pests of corn and specifically targets two major corn pests, *Helicoverpa zea* (corn earworm, CEW*)* and *Spodoptera frugiperda* (fall armyworm, FAW), but it is also effective against *Diatraea grandiosella* (southwestern corn borer, SWCB). Vip3Aa20 does not have insecticidal activity against *Ostrinia nubilalis* (European corn borer, ECB).

The Cry1Ab toxin expressed in *Bt*11 field corn is highly selective and very effective against ECB and SWCB. In addition, *Bt*11 is also effective against CEW and FAW. The modified Cry3A toxin expressed in MIR604 has insecticidal activity against two major coleopteran pests of corn, *Diabrotica barberi* Smith and Lawrence (northern corn rootworm, NCR) and *Diabrotica virgifera virgifera* LeConte (western corn rootworm, WCR), but no activity against lepidopteran pests.

On May 17, 2007, Syngenta submitted applications to register MIR162 maize, *Bt*11 x MIR162 corn, and *Bt*11 x MIR162 x MIR604 corn under Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). After conducting an initial science screen of all of the IRM data packages submitted with the applications, Biopesticides and Pollution Prevention Division (BPPD) noted two deficiencies, which were communicated to Syngenta in a letter dated October 30, 2007. Syngenta submitted a response letter on January 9, 2008. All aspects of these communications are provided in sections II(D)(1)(i)–(v) of this Biopesticides Registration Action Document (BRAD) chapter.

i. Deficiency #1

Syngenta has not provided sufficient data to determine the dose of the Cry1Ab toxin expressed in *Bt*11 corn and the Vip3Aa20 toxin expressed in MIR162 maize, independently and combined as *Bt*11 x MIR162 corn, versus *Diatraea grandiosella* (southwestern corn borer). Both the Cry1Ab toxin and the Vip3Aa20 toxin are active against *D. grandiosella*. Expression levels of Cry1Ab in *Bt*11 corn and *Bt*11 x MIR162 corn were noted as comparable by Syngenta, although these data have not been reviewed by BPPD. Syngenta did not discuss the relevance of these expression data to dose. The Environmental Protection Agency (EPA) requires data on the dose the plant provides of each of the plant-incorporated protectants (either singly or in combination) on all insect pests. Such data were not provided by Syngenta for Vip3Aa20 expressed in MIR162 maize or combined with *Bt*11 corn versus *D. grandiosella*, neither were they provided for Cry1Ab expressed in *Bt*11 x MIR162 corn. A high dose is defined as 25 times the protein concentration needed to kill susceptible larvae and is determined by the use of one or more of five imperfect methods to demonstrate that a transgenic crop expresses a high dose of insecticidal protein. Such data are needed to complete BPPD's technical review of Syngenta's proposed IRM strategy for *Bt*11 x MIR162 corn (and subsequently, *Bt*11 x MIR162 x MIR604 corn).

ii. Syngenta's Response #1

Scientific literature indicates that pyramiding a second plant-incorporated protectant with a currently registered PIP, both having independent activity against the same pest, will be beneficial for resistance management and can even allow for smaller refuge sizes than single protein events (Roush 1998; Caprio 1998; Zhao *et al*. 2003). Syngenta did not specifically discuss the dose of Vip3Aa20 in *Bt*11 x MIR162 corn for *D. grandiosella* because no decrease in refuge size was requested below the currently approved 20% non-*Bt* corn refuge for *D. grandiosella*. There is no scientific evidence to suggest that, by pyramiding an additional PIP with a currently registered PIP that expresses a novel protein which is active against the same pest, an existing refuge requirement will become unsuitable

for managing resistance in that pest with the pyramided hybrid. Regardless of the presence of Vip3Aa20 produced by MIR162, *Bt*11 x MIR162 corn has no systematic difference in Cry1Ab levels from *Bt*11 corn. Thus, the dose of Cry1Ab as produced by *Bt*11 for *D. grandiosella* is equivalent to that produced in *Bt*11 x MIR162 corn for *D. grandiosella*. Consequently, *Bt*11 x MIR162 corn will maintain the insecticidal activity and resistance management capabilities of *Bt*11 versus target pests and has the additional IRM benefit of producing Vip3Aa20 to protect maize plants further from other lepidopteran pests and resistance development.

iii. Deficiency #2

Simulation modeling did not consider *D. grandiosella* resistance to *Bt*11 x MIR162 corn.

iv. Syngenta's Response #2

Simulation computer modeling results have consistently shown that pyramiding two insecticidal proteins in the same plant that are active against the same pest will be beneficial for resistance management. It is important to note EPA's summary of the primary literature on pyramiding insecticidal proteins in its "Review of Dow AgroSciences' Product Durability (Insect Resistance Management) Plan in Support of the Section 3 Application for the Mycogen Brand Cry1F/Cry1Ac Construct 281/3006 Cotton" (Matten 2004). The Environmental Protection Agency states that "[p]revious modeling efforts by Roush (1998), Caprio (1998), and Zhao *et al*. (2003) have predicted that the durability of a two-gene stack will always be greater than a single-gene insect control protein." Regardless of the dose of Vip3Aa20 expressed by *Bt*11 x MIR162 corn, MIR162 will provide extra protection for delaying resistance when pyramided with *Bt*11 corn, *Bt*11 will provide extra protection for MIR162, and the existing 20% non-*Bt* corn refuge will suffice for delaying resistance development in *D. grandiosella* to *Bt*11 x MIR162 corn nationwide. The following points support the conclusion that the information provided in Syngenta's IRM volumes (Master Record Identification [MRID] Numbers 471374-07 and 471372-12; Kurtz *et al*. 2007a–b) is sufficient to support the registrations of *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn and that the proposed IRM plan for each product is scientifically valid:

- The EPA has already approved the IRM plan for *Bt*11 corn against *D. grandiosella*.
- Previous modeling data show that pyramids will always be more durable than single-gene PIPs.
- *Bt*11 x MIR162 corn produces Cry1Ab protein at levels comparable to *Bt*11 corn.
- Comparable levels of Cry1Ab in *Bt*11 x MIR162 corn equate to a comparable dose of Cry1Ab in *Bt*11 corn.

v. BPPD's Response to Syngenta's Letter Dated January 9, 2008

Southwestern corn borer is similar in biology to ECB; therefore, for this particular registration request of *Bt*11 x MIR162 corn with a 20% IRM refuge plan, BPPD assumes that the efficacy of the stack against SWCB is similar to its efficacy against ECB. At the moment, no additional modeling is required since no reduction in refuge size is requested. In the future, if Syngenta requests a reduction in refuge for *Bt*11 x MIR162 corn, BPPD would require dose data as well as additional simulation modeling for SWCB. However, field efficacy data for SWCB and/or a protein expression report are still recommended, perhaps as a condition of registration.

2. Pest Biology and Ecology

A summary of the biology and ecology for two major *Bt* corn lepidopteran target pests, the European corn borer and corn earworm, can be found in the IRM section of the Agency's *Bt* Crops Reassessment (U.S. EPA 2001a; *http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm*). In 2001, limited pest biology was available for the southwestern corn borer, fall armyworm, western corn rootworm, and northern corn rootworm. The BPPD IRM team reports additional biological information for these lepidopteran and coleopteran pest species.

i. Biology and Ecology of Southwestern Corn Borer

Host Range: Primary: corn

Life Cycle: Southwestern corn borer is multivoltine, occurring in the south-central United States (U.S.). Two generations per year are typically reported; three generations are sometimes possible. The active season for SWCB extends from May through harvest. This insect overwinters in its larval stage by tunneling into the base of the corn stalk. Pupation occurs with warming temperatures in spring. In the northern regions of its habitat, southwestern corn borer does not overwinter particularly well. In these cases, the first generation of SWCB will often be small followed by a larger second generation. Some dispersal by migration (older females) is thought to occur and contribute to periodic extensions of SWCB habitat. The life cycle mirrors another stalk-boring Lepidopteran, the European corn borer.

Larval: For the most part, southwestern corn borer larvae remain on their host plant with little interplant movement within the field.

Feeding: The feeding behavior of SWCB is substantially similar to ECB. First-generation larvae feed inside the whorl on foliage and can cause "dead-heart" injury. This destruction of the whorl can cause total loss of yield for the plant. Older larvae move down the plant and tunnel into the bottom two-thirds of the stalk, similar to ECB. Second-generation larvae cause the most severe damage due to both population

dynamics and feeding behavior. As mentioned above, the second-generation larvae feed in the leaf axils but also will feed on the primary ears between husks. Older larvae will move to the bottom of the plant in preparation of overwintering and tunnel into the stalk, often girdling the plant at the base. This damage is quite destructive and readily causes stem breakage.

Mating: Similar to ECB (see discussion in the Agency's 2001 *Bt* Crops Reassessment)

Oviposition: Eggs are laid singly or in groups of two to five on upper and lower leaf surface.

ii. Biology and Ecology of Fall Armyworm
(Nagoshi and Meagher 2004)

Host Range: Primary: corn (sweet and field), sorghum, rice, and grasses

Life Cycle: Fall armyworm is multivoltine throughout most of the U.S. and has two to six generations per year throughout the Corn Belt.

The active season for FAW on corn is later in the season from mid-June until harvest. The insect overwinters most commonly in the pupal stage in the soil about 20 millimeters (mm) underground, although other life stages such as the larva and adult may also overwinter. Fall armyworm pupae are not cold resistant, and in most winters, only southernmost populations in the Gulf Coast states survive winter. Populations north of the Gulf Coast are reestablished annually through progressive migrations of overwintering southern adults. Due to the nature of migration, fall armyworm often do not arrive until later in the summer where it can pose threat to late plantings of corn and sorghum.

Larval: After larvae hatch, they feed gregariously on the remnants of the egg mass and then disperse within several days. All larvae are mobile and will readily move to other plants in search of food. Older larvae may move *en masse* to other fields if they are in need of host plants. Fall armyworm larvae will tolerate the presence of other larvae on the same host, and multiple larvae on the same plant are not uncommon.

Feeding: Hatching larvae feed on the egg mass remains before dispersing within the plant or to other suitable plants. Small larvae on corn typically move to the whorl and feed on emerging foliage.

Mating: Pheromones may play a role in female mate selection. However, temporal partitioning could lead to assortative mating between strains of different host plants as well (i.e., corn-strain females call earlier than rice-strain females). In addition, strain-

specific mating has been observed to occur at opposite times of the night with no overlap.

Oviposition: Females are attracted to grasses in and about corn fields and to young, pretassel-stage corn plants. Eggs are laid in clusters of 50–100, usually on the underside of leaves. Anywhere from 1,000–1,500 eggs can be oviposited by a single female. Emerging females often fly for miles before locating a site suitable for ovipositing.

iii. Biology and Ecology of Corn Rootworm (CRW)

(McCaffery *et al*. 2005; *http://www.ipm.uiuc.edu/fieldcrops/insects/corn_rootworm/factsheet.html; http://ianrpubs.unl.edu/insects/ec1563.htm*; *http://www.cropsci.uiuc.edu/faculty/mgray/publications/2001_Transgenic_Insecticidal_Cultivars.pdf*)

Host Range: Primary: corn and some grasses

Life Cycle: Western corn rootworm and northern corn rootworm have similar life cycles. Insects are univoltine with larvae present from May through July. Adults are abundant from July through September. Rootworm larvae can complete development only on corn and a few other grassy species.

Larval: Mature larvae of the WCR are approximately one-half inch in length, while larvae of the NCR are approximately one-quarter inch in length. Larvae of both species generally hatch in May, but hatching may vary due to temperature differences and occurs later in northern latitudes as compared to southern latitudes of the U.S. (note that means emergence of WCR adults appears to be delayed by about six days in MIR604 corn as compared to non-treated corn).

Feeding: After larvae hatch, they begin to feed on root hair of corn plants and later tunnel inside roots. Larvae go through three instars before they begin pupation. Adult CRW feed on pollen and green silk of later planted corn fields and pollen of soybeans and alfalfa.

Mating: Females remain in the fields from which they emerged, while a small portion of males has been shown to leave native patches; mating occurs primarily within fields rather than between fields. Males emerge three to four days before females, and mating occurs shortly after females are present. Limited long-distance dispersal in adult females can occur but mostly in mated and preovipositional females.

Oviposition: Western corn rootworm females need to feed for approximately two weeks before they are able to lay eggs. During late summer, they oviposit an average of 500 eggs over several weeks in clutches of approximately 80 eggs in upper soil layers (oviposition ranges from 6 inches–12 inches in depth). This has been found to occur

in corn fields but also soybeans in east-central Illinois for WCR only. Females of the NCR are less likely to lay their eggs below an eight-inch depth. Both NCR and WCR overwinter in the egg stage. Some eggs can remain dormant up to several years, which may render crop rotation less effective as a tool to control CRW.

3. Dose

The determination of dose, or the amount of toxin expressed by the transgenic crop relative to the susceptibility of the target pests, is a critical component of IRM. Models have shown that a high dose of toxin coupled with a non-transgenic refuge to provide a supply of susceptible insects is the most effective strategy for delaying resistance in *Bt* crops. The high dose/refuge strategy assumes that resistance to *Bt* is recessive and is conferred by a single locus with two alleles resulting in three genotypes: susceptible homozygotes (SS), heterozygotes (RS), and resistant homozygotes (RR). The high dose/refuge strategy also assumes that initial resistance allele frequency will be low and extensive random mating between resistant and susceptible adults will occur. In practice, a high-dose PIP should express sufficient quantities of toxin to kill all susceptible insects (SS) as well as heterozygous insects with one resistance allele (RS). Lower dose PIPs might allow for survival of insects with at least one susceptibility allele (SS or RS), and effective IRM may still be possible with a suitable refuge strategy. To be able to demonstrate high dose, it is recommended that registrants generate data by at least two of the five laboratory and field approaches as outlined by the 1998 Scientific Advisory Panel (SAP) (U.S. EPA 1998) and described by the Agency in the 2001 *Bt* Crops Reassessment (U.S. EPA 2001a).

The 1998 SAP defined high dose as a level of toxin 25 times greater than is needed to kill all susceptible insects. The SAP also outlined five techniques to determine high dose:

(1) Serial dilution bioassay with artificial diet containing lyophilized tissues of *Bt* plants (tissue from non-*Bt* plants serving as controls);
(2) Bioassays using plant lines with expression levels approximately 25-fold lower than the commercial cultivar (determined by quantitative enzyme-linked immunosorbent assay [ELISA] or some more reliable technique);
(3) Survey large numbers of commercial plants in the field to make sure that the cultivar is at the $LD_{99.99}$ or higher to assure that 95% of heterozygotes would probably be killed (see Andow and Hutchison 1998);
(4) Similar to #3 above, but would use controlled infestation with a laboratory strain of the pest that had an LD_{50} value similar to field strains;
(5) Determine if a later larval instar of the targeted pest could be found with an LD_{50} that was about 25-fold higher than that of the neonate larvae. If so, the later stage could be tested on the *Bt* crop plants to determine if 95% or more of the later stage larvae were killed.

It must be noted that both the high dose definition and verification techniques were developed in 1998 when all of the registered *Bt* crops were single-toxin products targeted against lepidopteran pests. In recent years, PIPs in *Bt* corn have been approved that contain two genes targeted at the same insect pest. These "pyramided" products can be beneficial for IRM since target pests must overcome two toxins to develop field resistance to the PIP. The benefits are greatest for two toxins with unrelated modes of action (i.e., binding to different *Bt* receptor sites in the midgut) that are expressed at high doses in the plant (Roush 1994; Roush 1998).

For pyramided products, the dose of each toxin should be evaluated separately. This can be easily accomplished if the pyramided product is created through conventional breeding—in this case, the dose of the single-toxin products has already been established and the combined dose in the pyramided PIP can be determined with comparative efficacy studies. However, for pyramids created by non-conventional breeding (e.g., recombinant deoxyribonucleic acid [DNA] techniques), defining the dose can be more complicated since single-toxin lines may not be available (or commercialized) for comparisons. The dual toxins can also be evaluated collectively to determine an "effective high dose." In some examples, each toxin by itself may not supply a high dose but in combination a sufficient control (>95% of heterozygotes) is provided and can be considered high dose.

To evaluate dose, Syngenta conducted laboratory and field studies to demonstrate the dose status of *Bt*11 x MIR162 corn and its components, *Bt*11 corn and MIR162 maize. Two sets of experiments were conducted for FAW, CEW, and ECB: (1) laboratory bioassays using lyophilized *Bt*11 corn, MIR162 maize, *Bt*11 x MIR162 corn, and control plant tissue incorporated and diluted into insect diet to determine target pest susceptibility and (2) field tests on *Bt*11 corn, MIR162 maize, *Bt*11 x MIR162 corn, and control plants using controlled artificial infestation techniques during the 2006 growing season.

i. Verification Method #1, Results and Discussion

a. Bt11 High Dose Methodology and Results for Fall Armyworm

Tests were performed at Syngenta Seeds, Incorporated's Research Center in Minnesota (MN). Seed sources used in the assays were the same as the other location (mentioned under CEW and ECB) and the other insect species. Three transgenic corn hybrids (*Bt*11, MIR162, and *Bt*11 x MIR162; 42–45 plants each) and a non-transgenic negative control were greenhouse grown and provided the leaves for lyophilization.

Three trials with different concentrations (4% by weight = 25-fold dilution, 2% by weight = 50-fold dilution, and 1% by weight = 100-fold dilution) per transgenic treatments and one negative control were established in commercially available FAW meridic diet. Samples sizes ranged from 40 to 60 neonate larvae (1 larva per well); three total experiments were conducted over time to ensure repeatability of results. Dead larvae were recorded starting between day 10 and 12 and then every 2 to 4 days until all larvae were dead or no more mortality occurred in the 25x dilution wells. If

mortality did not reach 100% in transgenic treatments, mortality in the transgenic treatments was corrected using Abbott's method.

BPPD notes that *Bt*11 corn does not express a high dose with this method and has very little activity against FAW as is apparent by percentage mortality reported under method #1; mean corrected mortality ranged from 1.4% at the 100x dilution to 5.7% at the 25x dilution.

Table 1. *Bt*11 Mortality Results for FAW Using Lyophilized Tissue Bioassays

Test Material	Lyophilized Dilution	Mean Mortality % (Test 1)	Mean Mortality % (Test 2)	Mean Mortality % (Test 3)	Mean Observed or Corrected Mortality %
Negative control	25X	2.3	0	4.2	2.1
*Bt*11	25X	17.0	2.1	4.1	5.7[1]
*Bt*11	50X	5.8	2.1	2.0	1.2[1]
*Bt*11	100X	4.4	0	0	1.4

[1] Mean corrected mortality

b. MIR162 High Dose Methodology and Results for Fall Armyworm

For methodology, refer to procedures used for *Bt*11 and FAW in section II(D)(3)(i)(a) of this chapter.

BPPD agrees with Syngenta's conclusion. Results support that MIR162 maize expresses a high dose against FAW under method #1; mean corrected mortality ranged from 80.9% at the 100x dilution to 100% at the 25x dilution.

Table 2. MIR162 Mortality Results for FAW Using Lyophilized Tissue Bioassays

Test Material	Lyophilized Dilution	Mean Mortality % (Test 1)	Mean Mortality % (Test 2)	Mean Mortality % (Test 3)	Mean Observed or Corrected Mortality %
Negative control	25X	2.3	0	4.2	2.1
MIR162	25X	**100**	**100**	**100**	**100**
MIR162	50X	100	91.6	91.8	94.2[1]
MIR162	100X	88.6	79.2	76.6	80.9[1]

[1] Mean corrected mortality

c. Bt11 x MIR162 High Dose Methodology and Results for Fall Armyworm

For methodology, refer to procedures used for *Bt*11 and FAW in section II(D)(3)(i)(a) of this chapter.

BPPD agrees with Syngenta's conclusion. Results support that *Bt*11 x MIR162 corn expresses a high dose against FAW under method #1; mean corrected mortality ranged from 88.2% at the 100x dilution to 100% at the 25x dilution.

Table 3. *Bt*11 x MIR162 Mortality Results for FAW Using Lyophilized Tissue Bioassays

Test Material	Lyophilized Dilution	Mean Mortality % (Test 1)	Mean Mortality % (Test 2)	Mean Mortality % (Test 3)	Mean Observed or Corrected Mortality %
Negative control	25X	2.3	0	4.2	2.1
Bt11xMIR162	25X	**100**	**100**	**100**	**100**
Bt11xMIR162	50X	97.7	98.2	100	98.6[1]
Bt11xMIR162	100X	83.0	96.0	86.0	88.2[1]

[1] Mean corrected mortality

d. Bt11 High Dose Methodology and Results for Corn Earworm

Tests were performed at Syngenta Seeds, Incorporated's Research Center in Iowa (IA). Seed sources used in the assays were the same as the other location (mentioned under FAW) and the other insect species. Three transgenic corn hybrids (*Bt*11, MIR162, and *Bt*11 x MIR162; 330–440 plants each) and a non-transgenic negative control were greenhouse grown at each location and provided the silk material for lyophilization.

Three trials with different concentrations (4% by silk weight = 25-fold dilution, 2% by silk weight = 50-fold dilution, and 1% by silk weight = 100-fold dilution) per transgenic treatments and one negative control were established in commercially available CEW meridic diet. Sample sizes were 50 wells per treatment with 1 neonate larva per well; three total experiments were conducted over time to ensure repeatability of results. Dead larvae were recorded daily until all larvae were dead or no more mortality occurred in the 25x dilution wells. If mortality did not reach 100% in transgenic treatments, mortality in the transgenic treatments was corrected using Abbott's method.

BPPD agrees with Syngenta's conclusion. Results support that *Bt*11 corn does not express a high dose against CEW under method #1; mean corrected mortality ranged from 19.1% at the 100x dilution to 64.3% at the 25x dilution. There was a relatively high mortality in the control treatment, which indicates the presence of some non-controlled effects.

Table 4. *Bt*11 Mortality Results for CEW Using Lyophilized Tissue Bioassays

Test Material	Lyophilized Dilution	Mean Mortality % (Test 1)	Mean Mortality % (Test 2)	Mean Mortality % (Test 3)	Mean Observed or Corrected Mortality %
Negative control	25X	27.3	28.0	24.0	26.6
*Bt*11	25X	75.5	70.0	76.0	64.3[1]
*Bt*11	50X	64.0	52.0	68.0	47.3[1]
*Bt*11	100X	44.0	28.0	50.0	19.1[1]

[1] Mean corrected mortality

e. MIR162 High Dose Methodology and Results for Corn Earworm

For methodology, refer to procedures used for *Bt*11 and CEW in section II(D)(3)(i)(d) of this chapter.

There was high mortality in the negative controls ranging from 24% to 28%, which implies that the mortality observed in MIR162 transgenic treatments was not caused by treatment effects alone and is confounded by other non-controlled effects. Mean mortality (at 25x dilution) reported by the three independent tests ranged from 66% to 82%. BPPD notes that due to higher than preferred control mortality (≤ 28%), MIR162 maize appears to be less efficacious against CEW than reported by Syngenta. Regardless of control mortality, this method did not demonstrate high dose for MIR162 maize and CEW.

Table 5. MIR162 Mortality Results for CEW Using Lyophilized Tissue Bioassays

Test Material	Lyophilized Dilution	Mean Mortality % (Test 1)	Mean Mortality % (Test 2)	Mean Mortality % (Test 3)	Mean Observed or Corrected Mortality %
Negative control	25X	27.3	28.0	24.0	26.6
MIR162	25X	66.0	92.0	82.0	72.7[1]
MIR162	50X	52.0	80.0	66.0	53.7[1]
MIR162	100X	50.0	48.0	62.0	36.4[1]

[1] Mean corrected mortality

f. Bt11 x MIR162 High Dose Methodology and Results for Corn Earworm

For methodology, refer to procedures used for *Bt*11 and CEW in section II(D)(3)(i)(d) of this chapter.

There was a higher than preferred mortality in the negative controls ranging from 24% to 28%, which implies that the mortality observed in the MIR162 pyramided treatments was not caused by treatment effects alone and is confounded by other non-controlled effects. BPPD concludes that *Bt*11 x MIR162 corn likely expresses an "effective high dose" for CEW.

Table 6. *Bt*11 x MIR162 Mortality Results for CEW Using Lyophilized Tissue Bioassays

Test Material	Lyophilized Dilution	Mean Mortality % (Test 1)	Mean Mortality % (Test 2)	Mean Mortality % (Test 3)	Mean Observed or Corrected Mortality %
Negative control	25X	27.3	28.0	24.0	26.6
*Bt*11 x MIR162	25X	**100**	**100**	**100**	**100**
*Bt*11 x MIR162	50X	78.0	100	100	90.5[1]
*Bt*11 x MIR162	100X	68.0	64.0	66.0	55.8[1]

[1] Mean corrected mortality

g. Bt11 High Dose Methodology and Results for European Corn Borer

Tests were performed at Syngenta Seeds, Incorporated's Research Center in IA. Seed sources used in the assays were the same as the other location (mentioned under FAW) and the other insect species. Three transgenic maize hybrids (*Bt*11, MIR162, and *Bt*11 x MIR162; 330–440 plants each) and a non-transgenic negative control were greenhouse grown at each location and provided the leaves for lyophilization.

Three trials with different concentrations (4% by weight = 25-fold dilution, 2% by weight = 50-fold dilution, and 1% by weight = 100-fold dilution) per transgenic treatments and one negative control were prepared in General Lepidoptera diet from BioServ. Sample sizes were 10 plates with 5 neonate larvae each; three total experiments were conducted over time to ensure repeatability of results. Dead larvae were recorded daily until all larvae were dead or no more mortality occurred in the 25x dilution wells. If mortality did not reach 100% in transgenic treatments, mortality in the transgenic treatments was corrected using Abbott's method.

There was a higher mortality in the negative controls (10% to 12%) than is preferred by the Agency, which may imply that the mortality observed in *Bt*11 transgenic treatments may not be caused by treatment effects alone and is confounded by other non-controlled effects. However, 100% mortality at the 25x dilution provides strong evidence for a high dose in *Bt*11 corn against ECB.

Table 7. *Bt*11 Mortality Results for ECB Using Lyophilized Tissue Bioassays

Test Material	Lyophilized Dilution	Mean Mortality % (Test 1)	Mean Mortality % (Test 2)	Mean Mortality % (Test 3)	Mean Observed or Corrected Mortality %
Negative control	25X	10.0	9.8	12.0	10.6
*Bt*11	25X	**100**	**100**	**100**	**100**
*Bt*11	50X	68.0	72.0	60.0	54.6[1]
*Bt*11	100X	50.0	50.0	36.0	25.5[1]

[1] Mean corrected mortality

h. MIR162 High Dose Methodology and Results for European Corn Borer

For methodology, refer to procedures used for *Bt*11 and ECB in section II(D)(3)(i)(g) of this chapter.

BPPD agrees with Syngenta that MIR162 maize does not express a high dose and has very little efficacy against ECB. Furthermore, control mortality in the experiments was slightly higher than desirable, which suggests that mortality in MIR162 transgenic treatments may be confounded by other non-controlled effects and actual efficacy of MIR162 maize against ECB may be lower than results suggest.

Table 8. MIR162 Mortality Results for ECB Using Lyophilized Tissue Bioassays

Test Material	Lyophilized Dilution	Mean Mortality % (Test 1)	Mean Mortality % (Test 2)	Mean Mortality % (Test 3)	Mean Observed or Corrected Mortality %
Negative control	25X	10	8.0	12.0	10.0
MIR162	25X	10.0	12.0	10.0	10.7
MIR162	50X	4.0	0	10.0	4.7
MIR162	100X	4.0	12.0	4.0	6.7

i. Bt11 x MIR162 High Dose Methodology and Results for European Corn Borer

For methodology, refer to procedures used for *Bt*11 and ECB in section II(D)(3)(i)(g) of this chapter.

There was a slightly higher mortality in the negative controls (10% to 12%) than is preferred by the Agency, which implies that the mortality observed in *Bt*11 x MIR162 transgenic treatments may not be caused by treatment effects alone and is confounded by other non-controlled effects. However, 100% mortality at a 25x dilution provided sufficient evidence for a high dose determination in *Bt*11 x MIR162 corn against ECB.

Table 9. *Bt*11 x MIR162 Mortality Results for ECB Using Lyophilized Tissue Bioassays

Test Material	Lyophilized Dilution	Mean Mortality % (Test 1)	Mean Mortality % (Test 2)	Mean Mortality % (Test 3)	Mean Observed or Corrected Mortality %
Negative control	25X	10.0	9.8	12.0	10.6
*Bt*11 x MIR162	25X	**100**	**100**	**100**	**100**
*Bt*11 x MIR162	50X	80.0	80.0	68.0	68.8[1]
*Bt*11 x MIR162	100X	58.0	54.0	48.0	39.3[1]

[1] Mean corrected mortality

ii. Verification Method #4, Results and Discussion

In 2006, each pest was tested in separate trials at two locations (Iowa, Minnesota). At each location and within each trial, one non-replicated block of four treatments was grown (*Bt*11 corn, MIR162 maize, *Bt*11 x MIR162 corn, and control); between 50 and 655 plants were grown for controls and transgenic treatments. All FAW eggs were provided by Syngenta Seeds, Incorporated in MN; corn earworm larvae were provided by two labs, Syngenta Seeds, Incorporated in IA and French Agricultural Research, Incorporated in MN; and ECB eggs were provided by one lab, Syngenta Seeds, Incorporated in IA. The number of neonate larvae applied to plants was constant within but not across trials and locations (77 and 75 FAW neonates/plant, 20 and 20 CEW neonates/plant, and 163 and 210 ECB neonates/plant in IA and MN, respectively). Leaf damage and larval survival for FAW were assessed as early as 10 days after the final infestation to prevent significant plant-to-plant migration; ear damage and survivors for CEW were assessed as early as 15 days after the infestation

before larvae exited ears to pupate; European corn borer ear and stalk damage and survivors were assessed as early as 49 days after the infestation.

a. Fall Armyworm (FAW)

Control: A minimum of fifty random samples of plants were evaluated for FAW larvae at both locations. The number of insects observed on control plants in IA and MN was 67 and 222, respectively. The number of survivors per plant was much greater in MN than in IA.

*Bt*11 Corn: A minimum of fifty random samples of plants were evaluated for FAW larvae at both locations because very little activity against FAW was expected by *Bt*11 corn. The number of larvae observed in IA and MN was 47 and 39, respectively. The number of survivors per plant was greater in MN than in IA.

MIR162 Maize: The total number of plants assessed in IA and MN was 604 and 638, respectively; at both locations, no survivors were found. The results suggest that MIR162 maize expresses a high dose against FAW under method #4.

*Bt*11 x MIR162 Corn: The total number of plants assessed in IA and MN was 607 and 655, respectively; at both locations, no survivors were found. The results suggest that *Bt*11 x MIR162 corn expresses a high dose against FAW under method #4.

b. Corn Earworm (CEW)

Control: A random sample of approximately 100 plants each was evaluated for CEW larvae at both locations. The number of insects observed on control plants in IA and MN was 184 and 102, respectively. The number of survivors per plant appeared to be similar in both locations.

*Bt*11 Corn: The total number of plants assessed in IA and MN was 403 and 100, respectively. The number of larvae observed in IA and MN was 424 and 26, respectively. The results suggest that *Bt*11 corn has some activity but does not express high dose against CEW under method #4.

MIR162 Maize: The number of plants assessed in IA and MN was 348 and 426, respectively. The number of larvae observed in IA and MN was 10 and 2, respectively. The results suggest that MIR162 maize has very good activity, at least "near high dose," against CEW under method #4.

*Bt*11 x MIR162 Corn: The total number of plants assessed in IA and MN was 409 and 440, respectively; at both locations, no survivors were found. The results suggest that B*t*11 x MIR162 corn expresses an "effective high dose" against CEW under method #4.

c. European Corn Borer (ECB)

Control: A random sample of 100 and 50 plants was evaluated for ECB larvae at both locations. The number of insects observed on control plants in IA and MN was 75 and 125, respectively. The number of survivors per plant was higher in MN (2.5 survivors per plant) than in IA (0.75 survivor per plant).

*Bt*11 Corn: The total number of plants assessed in IA and MN was 501 and 600, respectively; at both locations, no survivors were found. The results suggest that *Bt*11 corn expresses a high dose against ECB under method #4.

MIR162 Maize: The total number of plants assessed in IA and MN was 100 and 50, respectively; the number of survivors found in IA and MN was 85 and 90, respectively, and compares to the number of survivors found on control plants. Results indicate that MIR162 maize does not have any activity against ECB.

*Bt*11 x MIR162 Corn: The total number of plants assessed in IA and MN was 650 and 601, respectively; at both locations, no survivors were found. The results suggest that *Bt*11 x MIR162 corn expresses a high dose against ECB under method #4.

iii. BPPD's Conclusions on High Dose

To be able to demonstrate high dose, registrants are required to provide data generated by **at least two** of the five laboratory and field approaches as outlined by the 1998 SAP (U.S. EPA 1998) and described by the Agency in the 2001 *Bt* Crops Reassessment (U.S. EPA 2001a). The BPPD IRM team's conclusions regarding the activity of the pyramid, *Bt*11 x MIR162 corn, are based on the review of dose data from verification methods #1 and #4 submitted in Syngenta's IRM chapter (MRID Number [No.] 471374-07) and are summarized below. For BPPD's high dose conclusions with respect to the single events, pyramided event (*Bt*11 x MIR162 corn), and verification methods, Table 10 can also be consulted.

- *Bt*11 x MIR162 corn expresses a high dose against FAW.
- *Bt*11 x MIR162 corn expresses a high dose against ECB.
- *Bt*11 x MIR162 corn expresses an "effective high dose" against CEW under verification method #4 only. Under verification method #1, *Bt*11 x MIR162 corn expresses a "probable effective high dose" based on three independent tests (n = 50 for each trial). Based on what is known about CEW and its high variability in response to toxins, it is questionable whether such a result can be consistently replicated.

- MIR162 maize alone has no activity against ECB.

- MIR162 maize does not express a high dose against CEW but may express a "near high dose."
- MIR162 maize expresses a high dose against FAW.

The activity and efficacy of *Bt*11 corn against some major pests has been assessed previously (U.S. EPA 2001a). However, because *Bt*11 corn is one of the events in the MIR162 pyramid, new efficacy data had to be submitted for the Section 3 registration. BPPD's conclusions about these data are as follows:

- *Bt*11 corn has low activity against FAW.
- *Bt*11 corn does not express a high dose against CEW.
- *Bt*11 corn expresses a high dose against ECB.

Table 10. BPPD's High Dose Determination for *Bt*11 Corn, MIR162 Maize, and Their Combined Event, *Bt*11 x MIR162 Corn, Against Lepidopteran Pests Based on Experimental Data Provided by Syngenta

Species	Method 1			Method 4		
	***Bt*11**	**MIR162**	***Bt*11 x MIR162**	***Bt*11**	**MIR162**	***Bt*11 x MIR162**
FAW	No high dose	High dose	High dose	Low activity	High dose	High dose
CEW	No high dose[1]	No high dose[1]	Probable effective high dose	No high dose	Near high dose	Effective high dose
ECB	High dose	No activity	High dose	High dose	No activity	High dose

* Shaded fields indicate high dose determinations by BPPD for the single toxins or pyramided *Bt* product.
[1] Control mortality was in excess of 10% and as high as 28%; thus, the Agency's dose conclusions are more conservative and may differ from Syngenta's reported conclusions.

4. Cross-Resistance Potential

*Bt*11 x MIR162 corn is the second *Bt* corn product with pyramided lepidopteran-active traits. There are also pyramided lepidopteran-active products available in cotton already (i.e., Bollgard II®, VipCot™, and Widestrike®). While these stacks in cotton express two different Cry proteins, *Bt*11 x MIR162 corn expresses two completely unrelated insecticidal proteins, a crystal protein and a vegetative insecticidal protein. In their submission requesting registration for *Bt*11 x MIR162 x MIR604 corn, Syngenta provided data and discussed the potential for cross-resistance for CEW since it is a pest of both corn and cotton in the United States. Thus, cross-resistance between similar Cry toxins and Cry toxins and Vip3A is of concern. Cross-resistance potential for ECB was not addressed since the pest is not susceptible to Vip3A. Southwestern corn borer has a similar biology as ECB; therefore, in absence of any dose data, BPPD assumes per this registration request for a 20% refuge that SWCB has a similar response to the two toxins as ECB. Fall armyworm is susceptible to Vip3A but does not show much susceptibility to Cry1Ab.

Analyses of resistance to *Bt* Cry proteins indicate that cross-resistance occurs most often with proteins that are similar in structure (Tabashnik 1994; Gould *et al*. 1995). While direct structural

information of the Vip3A protein is missing (Lee *et al*. 2003), this novel *Bt* protein does not share any sequence homology with the known *Bt* Cry protein genes, and the predicted secondary structure gives no indication of a similar domain organization or alpha-helical bundle region within the polypeptide sequence of Vip3A as exists for the Cry proteins. Protein-folding blasts reveal that Vip3A may be a pore-forming protein that has a structure of beta-barrels (Syngenta unpublished data). To investigate further the potential for cross-resistance of Vip3A to Cry proteins, Syngenta examined the mode of action of Vip3A at selected steps critical to the mode of action of *Bt* Cry proteins: proteolytic activation, receptor binding, and pore forming.

i. Proteolytic Activation

Vip3A protein activation studies have shown that proteolysis occurs in the midgut of both susceptible and non-susceptible insects. These data suggest that proteolytic activation is not a key factor in insect toxicity and specificity. Further studies have shown that there are similarities between how Vip3A, Cry1Ac, and Cry2Ab are processed; all three toxins are activated by trypsin or gut juice extracts (Lee *et al*. 2006). Therefore, a small but theoretical risk of cross-resistance between these toxins exists at this step.

ii. Receptor Binding

Several studies (receptor binding, competition binding, ligand-blotting assays) in the tobacco hornworm (*Manduca sexta*), corn earworm (*Helicoverpa zea*) and tobacco budworm (*Heliothis virescens*) have shown that receptors for Vip3A are distinct from those of Cry1Ab, Cry1Ac, and Cry2Ab. In these studies, Vip3A did not bind to APN and cadherin-like proteins, which are known to be Cry1A receptors. Cry2Ab appears to have non-specific binding properties; nonetheless, in competition-binding assays, results indicate that Vip3A does not share binding sites with Cry2Ab. BPPD concurs with Syngenta that the risk of cross-resistance should be minimal between Vip3A and Cry1Ac/b and Vip3A and Cry2Ab2 based on receptor-binding studies.

iii. Pore Forming

The pore-forming properties of Vip3A are unique in that the kinetics of Vip3A pore formation are more than eight times slower than for equimolar Cry1Ab; pore channels are characterized by long, open times and a predominantly open state; and stable channels formed by Vip3A differ considerably in their conductance state and cation specificity from Cry1A protein. In addition, Domain I, modulated by Domain III interactions, has been considered responsible for the pore-formation steps in the *Bt* Cry protein mode of action. Again, direct structural information is not available for the Vip3A protein. Yet, available information gives no indication of a similar domain organization or alpha-helical bundle region within the polypeptide sequence as exists for the Cry proteins. BPPD agrees with Syngenta that the risk of cross-resistance between Vip3A and Cry1A

proteins is minimal based on pore-forming studies, which show that channels formed by Vip3A are structurally and functionally distinct.

iv. BPPD's Conclusions on Cross-Resistance Potential

Based on the cross-resistance studies and background information available in the literature, BPPD agrees with Syngenta that the risk of cross-resistance should be minimal between the Vip3A and Cry1A toxins and the Vip3A and Cry2Ab toxins. Vip3A does not bind to APN and cadherin-like proteins and to Cry2Ab2 non-specific binding sites; thus, Vip3A does not share binding sites with Cry1A and Cry2Ab toxins. Additionally, Vip3A pore channels formed in the midgut of insects are structurally and functionally distinct from Cry1A-type proteins.

5. Modeling

The Environmental Protection Agency has used predictive models to compare IRM strategies for *Bt* crops. Because models cannot be validated without actual field resistance, models have limitations and the information gained from the use of models is only part of the weight of evidence used by EPA in assessing the risks of resistance development. It was the consensus of the 2000 SAP Subpanel (U.S. EPA 2001b) that models were an important tool in determining appropriate *Bt* crop IRM strategies. They agreed that models were "the only scientifically rigorous way to integrate all of the biological information available, and that without these models, the Agency would have little scientific basis for choosing among alternative resistance management options." They also recommended that models must have an agreed upon time frame for resistance protection. For example, conventional growers may desire a maximum planning horizon of five years, while organic growers may desire an indefinite planning horizon. The Subpanel recommended that model design should be peer-reviewed and parameters validated. Models should also include such factors as level of *Bt* crop adoption, level of compliance, economics, fitness costs of resistance, alternate hosts, spatial components, stochasticity, and pest population dynamics.

Syngenta proposed that a 20% refuge be used to manage insect resistance to *Bt*11 x MIR162 corn in cotton-growing areas rather than the current 50% structured refuge requirement for single-gene, lepidopteran-control products. The major pest of concern for *Bt* corn in the cotton-growing areas is CEW (also known as cotton bollworm [CBW] when it feeds on cotton), although ECB, FAW, and sugarcane borer (SCB) are also sporadic corn pests in cotton-growing areas. As outlined in the 2001 *Bt* Crops Reassessment (*http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm*), the cotton-growing areas where the 50% structured non-*Bt* corn refuge is a requirement include the following states: Alabama, Arkansas, Georgia, Florida, Louisiana, North Carolina, Mississippi, South Carolina, and some counties in Oklahoma, Tennessee, Texas, Virginia, and Missouri (for specific county listing, the 2001 *Bt* Crops Reassessment can be consulted).

Syngenta commissioned Dr. Michael Caprio to evaluate the risk of resistance evolving to *Bt*11 x MIR162 corn with a 20% refuge in cotton-growing regions. In the next few paragraphs, BPPD

summarizes the most important features and assumptions of the model, the scenarios modeled, and simulation results for CEW.

Dr. Caprio used a spatially explicit, stochastic, population genetic model incorporating parameter uncertainty (maximum/minimum value, most likely value, assuming normal distribution) and interaction; two loci, heterogeneous habitats (wild hosts, *Bt* and non-*Bt* corn, *Bt* cotton) with different toxin expression levels in different parts of corn plants; and pest biology/ecology. The model assumed that there were two lepidopteran-active *Bt* traits available for transgenic crops, a Vip3A trait and a Cry1Ab/c trait expressing a high dose for the Vip toxin and a moderate to high dose for the Cry toxin in corn and cotton. Both *Bt* proteins were expressed either in a single gene or in a pyramided product: Vip3A, Cry1Ab/c, VipCot™, and *Bt*11 x MIR162 corn. Dr. Caprio's simulation model incorporated crop utilization data from several studies that indicate that in the south-central U.S., corn earworm larvae feed on non-crop hosts such as red clover and geranium in spring, the following two generations feed on corn, and the next one to two generations move on to cotton and other crop hosts such as soybean and sorghum before getting ready to overwinter.

Several scenarios were modeled:

- Twenty-percent sprayed *cotton* non-*Bt* refuge with 80% VipCot™ and 50% sprayed *corn* non-*Bt* refuge with 50% *Bt*11 x MIR162 corn
- Twenty-percent sprayed *cotton* non-*Bt* refuge with 80% VipCot™ and 20% sprayed *corn* non-*Bt* refuge with 80% *Bt*11 x MIR162 corn
- A series of single-gene *Bt cotton* and *Bt corn* (Cry1A) planted along with VipCot™ cotton and *Bt*11 x MIR162 corn pyramids

i. Impact of Reducing the Non-*Bt* Refuge in Cotton-Growing Regions

When *Bt* corn refuge was reduced to 20% in the cotton-growing region and no single-gene crop was present, resistance did not evolve to either Cry1Ab or Vip3A. The simulations further suggest that within 25 years, there is little risk of CEW resistance evolving to the *Bt*11 x MIR162 pyramid whether 50% or 20% non-*Bt* refuge is planted in cotton-growing regions.

ii. Impact of Single-Gene Events on the Longevity of Stacked Events

In 80% of the simulations, resistance evolved to Cry1Ab during a 25-year period when a single-gene crop was planted. The more single-gene crop planted, the faster resistance evolved to Cry1Ab/c. When no single-gene crop was present, resistance did not evolve to either Cry1Ab or Vip3A.

iii. BPPD's Analysis of and Conclusions on Dr. Caprio's Model

Based on the simulation results with moderate to high-dose assumptions for *Bt*11 x MIR162 corn, Dr. Caprio concluded that reducing the structured non-*Bt* corn refuge in cotton-growing regions from 50% to 20% would not lead to increased risk of resistance in CEW to VipCot™ cotton and *Bt*11 x MIR162 corn during the 25-year time frame of the model. BPPD notes that Syngenta's dose results warrant a "near high dose" expression for Vip3A against CEW rather than a high dose but a "probable effective high dose" for the MIR162 pyramided product. It is not clear how sensitive modeling results are to the "dose parameter inputs" and how a slight change in dose parameter input value, in conjunction with a reduced refuge requirement in the cotton-growing regions, would affect CEW resistance.

In addition to Dr. Caprio's modeling efforts and results and before a conclusion regarding reduced corn refuge in the cotton-growing region can be warranted, further consideration needs to be given to (1) justification for the assumed crop patterns/host availability in the simulation model, (2) cross-resistance potential, and (3) dose for the single toxin and pyramided product. Stable isotope analysis of pheromone-trapped males from 1997–1999 support that CBW adults feed on a mix of C_3 (i.e., corn) and C_4 (i.e., sorghum and/or wild host) plants in the early season, while moths caught late in the season predominantly originate from C_4 hosts (Gould *et al*. 2002). In addition, host utilization data from the southern and southeastern U.S. (2002–2003) support that CBW larvae have been found predominantly on corn throughout the early and mid-season and on soybean, tobacco, cotton, corn, and sorghum throughout the later season (Jackson *et al*. 2008). The authors comment that these alternate host crops provided a stable refuge during the years investigated with United States Geological Survey (USGS)/National Agricultural Statistics Service (NASS) data (1995–2002). Thus, CBW moths are produced on alternative hosts in cotton-growing areas that may be available to mate with any putative-resistant CBW moths and further dilute resistance. In addition, cross-resistance data submitted by Syngenta demonstrate that risk of cross-resistance is minimal between the Vip3A and Cry1A toxins and the Vip3A and Cry2Ab toxins based on activation studies, receptor binding, competition binding, and ligand-blotting assays, as well as pore-forming studies (see section II(D)(4) of this chapter). Finally, the dose studies show that Vip3A has good activity and that the MIR162 pyramided product expresses an "effective high dose" (under method 4) against CEW (see section II(D)(3) of this chapter).

BPPD concludes that all the evidence together from the host utilization, cross-resistance, binding, and dose studies supports that a 20% non-*Bt* corn refuge for *Bt*11 x MIR162 corn in the southern cotton-growing areas would be sufficient to manage the risk of resistance evolution to *Bt* corn and *Bt* cotton products.

6. Refuge Strategy

The size, placement, and management of the refuge are critical to the success of the high dose/structured refuge strategy to mitigate insect resistance to *Bt* proteins produced in corn (as well

as cotton and potatoes). The 1998 SAP Subpanel defined structured refuges to "include all suitable non-*Bt* host plants for a targeted pest that are planted and managed by people." Furthermore, the Subpanel stated that "[t]hese refuges could be planted to offer refuges at the same time when the *Bt* crops are available to the pests or at times when the *Bt* crops are not available." The 1998 SAP Subpanel also suggested that a production of 500 susceptible adults in the refuge for every adult in the transgenic crop area (assuming a resistance allele frequency of 5×10^{-2}) would be a suitable goal. The placement and size of the structured refuge employed should be based on the current understanding of the pest biology data and the technology. The 2000 SAP Subpanel echoed the 1998 SAP's recommendations that the refuge should produce 500 susceptible insects to 1 resistant insect and that regional IRM working groups would be helpful in developing policies.

Syngenta submitted its reduced refuge request for *Bt* corn in cotton-growing regions for Event MIR162 maize. Syngenta states that their refuge planting options include separate fields, blocks within fields, and strips across fields. Generally, these refuge options are sufficient for the pyramided product, *Bt*11 x MIR162 corn, intended to be commercially marketed. BPPD recommends that the following refuge requirements be applied to *Bt*11 x MIR162 corn:

- Specifically, growers must plant a structured refuge of at least 20% non-*Bt* corn and/or non-lepidopteran-resistant *Bt* corn that may be treated with insecticides, as detailed below, to control lepidopteran stalk-boring and other pests.
- Refuge planting options include: separate fields, blocks within fields (e.g., along the edges or headlands), perimeter strips, and strips across the field.
- External refuges must be planted within ½ mile.
- When planting the refuge as strips across the field or as perimeter strips, refuges must be at least 4 consecutive rows wide.
- Insecticide treatments for control of ECB, CEW, SWCB, and other lepidopteran pests listed on the label, grower guides, or other educational material may be applied only if economic thresholds are reached for one or more of these target pests. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents or crop consultants). Instructions to growers will specify that microbial *Bt* insecticides must not be applied to non-*Bt* corn and/or non-lepidopteran-resistant *Bt* corn refuges.

Since the other MIR162 product to be commercially marketed (i.e., *Bt*11 x MIR162 x MIR604 corn) expresses modified Cry3A, refuge options are driven by the requirements for CRW refugia. The only CRW refuge options that are acceptable for *Bt*11 x MIR162 x MIR604 corn are in-field and adjacent (also common) refuge. No other CRW refuge option will be permissible because there is evidence of non-random mating for CRW between non-adjacent corn fields.

For clarity, BPPD restates the refuge planting options available to Syngenta for *Bt*11 x MIR162 x MIR604 corn. These options are taken out of the *Bt*11 x MIR604 corn (EPA Registration Number

67979-8) registration notice with some modifications (particularly in relation to the percentage refuge required) because *Bt*11 x MIR162 x MIR604 corn is both stacked for protection from lepidopteran and coleopteran pests and pyramided for protection from lepidopteran pests. Under the established refuge strategy for *Bt* corn stacked for corn borer and corn rootworm protection, growers can choose from two different planting options to fulfill IRM requirements. These options, which are briefly summarized below, include one shared, common refuge for both insect groups or separate refuges for each insect group.

Agency-approved common refuge option for *Bt* corn products stacked for corn borer and corn rootworm protection:

- Refuge is 20% of total corn acres (in this case, refuge can be reduced from 50% to 20% in the cotton-growing areas because *Bt*11 x MIR162 x MIR604 corn expresses two toxins that target lepidopteran pests).
- Refuge is planted directly next to or within the stacked *Bt* corn field.
- Refuge can be treated with a non-*Bt* foliar insecticide for control of late season pests, if pest pressure reaches an economic threshold for damage. If rootworm adults are present at the time of foliar applications, then the stacked *Bt* corn field must be treated in a similar manner.

Agency-approved separate refuge option for *Bt* corn products stacked for corn borer and corn rootworm protection:

- Twenty-percent refuge for corn rootworm planted immediately next to or within the stacked *Bt* corn field; *Bt* corn with a single lepidopteran trait may be planted in the refuge, but total acreage is not to exceed 80% of *Bt* lepidopteran corn acres.
- Rootworm refuge may be treated with a non-*Bt* foliar insecticide for control of late season lepidopteran pests. If adult rootworms are present at the time of foliar applications, the stacked *Bt* corn field must be sprayed as well.

- Twenty-percent refuge for corn borer (in this case, refuge is reduced from 50% to 20% in the cotton-growing areas because *Bt*11 x MIR162 x MIR604 corn expresses two toxins that target lepidopteran pests); *Bt* corn with a single corn rootworm trait may be planted in the refuge, but total acreage is not to exceed 80% of *Bt* rootworm corn acres.
- Corn borer refuge may be treated with a non-*Bt* foliar insecticide for corn borer control, if pest pressure reaches an economic threshold for damage. The stacked *Bt* corn field would not have to be sprayed under this option.

BPPD recommends that these specific details be applied to *Bt*11 x MIR162 x MIR604 corn.

7. Resistance Monitoring

Syngenta submitted a resistance monitoring program to the Agency for the MIR162 pyramid with *Bt*11 only. BPPD concludes that monitoring for CRW will continue as outlined in the mCry3A BRAD (U.S. EPA 2007). Furthermore, BPPD recommends that Syngenta continue to consider sublethal bioassays (head capsule measurements) and molecular marker methods for CRW monitoring in addition to mortality assays. Monitoring for the Cry1Ab toxin has been (and will continue to be) conducted under the *Bt*11 corn registration.

Syngenta will monitor for resistance and/or trends in decreased susceptibility to Vip3Aa20 in CEW. Syngenta has been working with Dr. Randy Luttrell since 2006 and 2007 to develop assay methods and baseline Vip3A susceptibility data, respectively. Syngenta mentions that it will monitor for resistance in SWCB but does not provide any information beyond that.

BPPD notes that Syngenta did not provide very much information about their collaborators and intended monitoring plans for Vip3A and SWCB. In order to facilitate future communication between BPPD and Syngenta, the IRM team makes the following recommendations for monitoring procedures:

- Use the diagnostic concentration (LC_{99}) for Vip3A if the approach has proven successful, the pest is susceptible to the toxin, and population variance is small.
- Conduct follow-up testing of larval survivors for all toxins where field population survivorship on a diagnostic concentration is significantly different from lab/reference colony's survivorship.
- Submit a final Vip3Aa20 monitoring plan for the major target pests (CEW and SWCB) as a condition of registration.

BPPD has the following recommendations for Syngenta specifically for CEW (but not only): If effort has been put into developing a discriminating or diagnostic concentration for CEW and Vip3A and the diagnostic concentration cannot be achieved due to high variability in response to the toxin, then a comparison in baseline susceptibility (i.e., LC_{50}s) may be a feasible approach to monitoring. Estimated LC_{50}s may serve well as a baseline-monitoring tool for shifts in susceptibility to *Bt* toxins; however, the LC_{50} approach is not useful in discriminating resistant from susceptible individuals. Therefore, this approach must be linked with follow-up testing of populations with elevated LC_{50}s relative to previously established baseline susceptibility.

8. Grower Education

Syngenta proposes to use the following methods, which have already been established for other registered PIPs, to educate growers:

- Purchasers of MIR162 stacked and/or pyramided products will sign a grower agreement.
- The grower agreement and/or stewardship documents, which are referenced in the grower agreement, will set forth terms of the current IRM program and contractually bind growers to comply with IRM requirements.
- An annual affirmation system for MIR162 stacked and/or pyramided product growers will ensure they understand that they are contractually bound to comply with IRM requirements.
- IRM educational material will be distributed to growers through written materials, in-person communication, and other media (i.e., internet).
- Sales personnel and seed distributors will be properly trained in order to provide another educational resource for growers.
- Educational efforts will be coordinated with other organizations.

In addition to Syngenta's proposed educational outreach program, BPPD requests that Syngenta perform the following actions:

(1) Within 90 days from product registration, submit a copy of the grower agreement/stewardship documents and written description of a system assuring that growers will sign a grower agreement.
(2) Revise and expand, as necessary, its education program to take into account information collected through the compliance survey.
(3) Maintain records of all signed MIR162 stacked and/or pyramided product grower agreements for three years.

BPPD concludes that the proposed program meets the Agency's requirement for grower education at this stage of the product registration process.

9. Compliance

Grower compliance with refuge and IRM requirements is a critical element for resistance management. Significant non-compliance with IRM among growers may increase the risk of resistance for *Bt* crops. To minimize the effects of non-compliance, it is necessary to develop a broad compliance program as part of the IRM strategy that includes the following elements: (1) an understanding of the effect of non-compliance on IRM, (2) identification of compliance mechanisms to maximize adoption of IRM requirements, (3) measurement of the level of compliance, and (4) establishment of an enforcement structure to ensure compliance and penalize non-compliance.

Syngenta has committed to implementing a compliance assurance program (CAP) designed to evaluate the extent to which growers of the MIR162 stacked and/or pyramided products are complying with the IRM requirements and take reasonable actions necessary to assure that non-compliant growers become compliant with those requirements. Consistent with the registration of other *Bt* corn PIPs, there are several key elements to the CAP that Syngenta commits to employ:

- A "phased compliance approach" outlining instances of non-compliance to IRM terms and options of responding to non-compliant growers, such as denying access to MIR162 technology.
- Annual survey conducted by a third party that will measure the degree of compliance by growers in different regions where the MIR162 stacked and/or pyramided products are grown.
- Survey that will obtain grower feedback on usefulness of educational tools and initiatives and provide understanding of any difficulties growers encounter with IRM requirements[a].
- Annual on-farm assessment followed by appropriate action consistent with the "phased compliance approach" for non-compliant growers.
- "Tips and complaints" line with follow-up investigations and appropriate actions taken consistent with the "phased compliance approach" for non-compliant growers.

BPPD concludes that Syngenta has included the major requirements needed by a compliance assurance program. Syngenta's proposed compliance assurance program resembles CAPs for other already registered *Bt* PIPs and meets the Agency's requirement at this stage of the product registration process. BPPD recommends that the compliance assurance program for the MIR162 stacked and/or pyramided products be harmonized with the compliance assurance programs already in place for previously registered *Bt* corn products.

10. Remedial Action Plan

Remedial action plans are a potential response measure should resistance develop to *Bt* crops. Since resistance may develop in "localized" pest populations, it may be possible to contain the resistance outbreak before it becomes widespread. A specific remedial action plan should clearly indicate what actions the registrant will take in cases of "suspected" resistance (i.e., unexpected damage) and "confirmed" resistance. The remedial action plan can also include appropriate adaptations for regional variation and appropriate stakeholders. To fully mitigate resistance, a critical element of any remedial action plan should be that once pest resistance is confirmed, sales of all *Bt* corn hybrids that express a similar protein or a protein in which cross-resistance potential has been demonstrated would cease in the affected region (*http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm*).

Syngenta states that it will take following actions if Cry1Ab and/or Vip3A resistance to any of the major target pests is "suspected":

- Expression levels in damaged plants will be measured to ascertain that they match expected levels for Cry1Ab and Vip3A.

[a] Syngenta proposes to revise and expand, as necessary, its compliance assurance program to take into account information collected through the compliance survey.

- Other reasonable causes will be investigated for crops that are damaged.
- Growers in affected region will be instructed to use alternate pest control measures for pests with suspected resistance and to destroy crop residues immediately after harvest.

Syngenta states that it will take the following actions if Cry1Ab and/or Vip3A resistance to any of the major target pests is "confirmed":

- Notify the Agency within 30 days of resistance confirmation.
- Notify affected customers and extension agents about confirmed resistance.
- Direct affected customers and extension agents to employ alternative control measures.
- Instruct customers and extension agents to incorporate crop residues into soil following harvest to minimize possibility of overwintering by resistant insects.
- Cease sale and distribution of MIR162 stacked and/or pyramided products in the affected area.
- Notify the Agency, within 90 days, of mitigation measures that were implemented.
- Provide the Agency, within 90 days, with a proposed long-term resistance management action plan for the affected area including elements such as information exchange with customers and extension agents, increased monitoring of target pests, and alternative measures to reduce or control target pests.

BPPD concludes that the steps outlined in the remedial action plan and the depth of detail provided are similar to remedial action plans for already registered *Bt* PIP products. Syngenta's remedial action plan meets the Agency's requirement for this stage of the product registration process. BPPD recommends that the remedial action plan for the MIR162 stacked and/or pyramided products be harmonized with the plans already in place for previously registered *Bt* corn products.

11. Reporting Requirements

If requested or required, Syngenta commits to meeting with the EPA to discuss results from the grower survey, results from the resistance monitoring program, and other relevant IRM plan issues. In addition, Syngenta will provide the following to the Agency by January 31st each year:

- annual sales summed by state (county level data available upon request)
- number of units of seed shipped/sold and not returned
- number of units of seed sold to persons with signed grower agreements
- final written summary of survey results for the prior year and survey plans for the upcoming year
- annual report summarizing activities and results of the CAP for the prior year and plans for the upcoming year
- substantive changes to the education program completed during the previous year

Insect resistance monitoring results will be provided to the Agency on August 31st of each year.

At this stage of the registration process for the MIR162 stacked and/or pyramided products, BPPD is satisfied with Syngenta's commitment to fulfill their reporting requirements.

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E. Benefits and EPA Public Interest Finding

Note: The information that follows in sections II(E)(2), II(E)(3)(i)(a), II(E)(3)(iii)(a), II(E)(3)(iv)(a), and II(E)(3)(v)(a) are near-verbatim excerpts taken from Syngenta's Public Interest Document (Ward and Vlachos 2007) and is not intended to represent the conclusions of the Environmental Protection Agency. Rather, this information is presented to provide insight into Syngenta's rationale for claiming that the registrations of MIR162, *Bt*11 x MIR162, and *Bt*11 x MIR162 x MIR604 are in the public interest.

1. Background

Syngenta's *Bt*11 x MIR162 x MIR604 corn is a stacked and pyramided transgenic corn trait that expresses the two registered crystal protein toxins, Cry1Ab and mCry3A, and incorporates the novel *Bacillus thuringiensis* (*Bt*) Vip3Aa20 toxin, which is 99.9% identical in amino acid sequence to the Vip3Aa19 toxin produced in COT102 cotton (which is a component of a registered Cry1Ab x Vip3Aa19 cotton product). Vip3A is different from Cry proteins as it is produced during vegetative growth of the bacteria, does not form parasporal crystal proteins, and is secreted (but not processed upon secretion) from the cell as a soluble protein. While its physical manifestations of intoxication in sensitive larvae resemble those of Cry proteins (gut paralysis and lysis of midgut epithelial cells) (Schnepf *et al*. 1998), activated Vip3A does not bind to the same receptors (aminopeptidase-N [APN] and cadherin-like receptors, in the case of Cry1Ab). These two types of *Bt* proteins (Vip3Aa, Cry1Ab) do not appear to share binding sites.

Syngenta received an experimental use permit (EUP) to allow field testing of the plant-incorporated protectant (PIP), Event MIR162 maize, and its combined-trait varieties, *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn, in 23 states to cover the period from March 1, 2007 through February 29, 2008. Event MIR162 maize expresses the Vip3Aa20 insect control protein. Vip3Aa20 has insecticidal activity against several lepidopteran pests of corn and specifically targets two major corn pests, *Helicoverpa zea* (corn earworm, CEW*)* and *Spodoptera frugiperda* (fall armyworm, FAW), but it is also effective against *Diatraea grandiosella* (southwestern corn borer, SWCB). Vip3Aa20 does not have insecticidal activity against *Ostrinia nubilalis* (European corn borer, ECB). The Cry1Ab toxin expressed in *Bt*11 field corn is highly selective and very effective against ECB and SWCB. In addition, *Bt*11 corn is also effective against CEW and FAW. The modified Cry3A expressed in MIR604 corn has insecticidal activity against two major coleopteran pests of corn, *Diabrotica barberi* Smith and Lawrence (northern corn rootworm, NCR) and *Diabrotica virgifera virgifera* LeConte (western corn rootworm, WCR), but no activity against lepidopteran pests.

The *Bt*11 maize (Cry1Ab) benefits have been previously discussed in the 2001 *Bt* Crops Reassessment (U.S. EPA 2001) and can be viewed online at: *http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm*.

Benefits resulting from the introduction of MIR604 corn (modified Cry3A) have been published in the Biopesticides Registration Action Document (BRAD) for mCry3A (U.S. EPA 2007) and can be viewed online at:
http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006509.pdf.

Matten (2007) reviewed the results of a small-scale field trial conducted at multiple locations during the 2005 corn-growing season. The review concluded that MIR162 maize provides significant crop protection against feeding damage caused by *Agrotis ipsilon* (black cutworm, BCW), FAW, CEW, and *Striacosta albicosta* (western bean cutworm, WBCW). The level of protection provided by MIR162 maize is significantly better than that provided by *Bt*11 alone or a negative isoline with a conventional insecticide standard. Small-scale field tests demonstrated that *Bt*11 x MIR162 pyramided hybrids controlled BCW, FAW, CEW, WBCW, and ECB. When MIR604 was combined with MIR162, there was some evidence of a possible synergistic effect in the control of corn rootworm in 2005.

In this BRAD chapter, Biopesticides and Pollution Prevention Division (BPPD) will discuss and present benefits resulting from the introduction of MIR162 maize and the stacked and/or pyramided *Bt* trait products, *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn, and its conclusions on Syngenta's efficacy studies for ECB, CEW, FAW, and WCR. Insecticidal efficacy of the pyramided product, *Bt*11 x MIR162 corn, against the major lepidopteran pests (ECB, CEW, and FAW) will also be discussed and compared to the efficacy of *Bt*11 x MIR162 x MIR604 corn to look for possible synergistic effects.

2. Syngenta's Executive Summary of Its Public Interest Document (PID)

Syngenta is seeking registration for a new plant-incorporated protectant, the Vip3Aa20 derived from *Bacillus thuringiensis*, as produced in maize transformation event MIR162. Syngenta is also seeking registration for two combined-trait maize cultivars containing MIR162 and two other registered plant-incorporated protectants, Cry1Ab in *Bt*11 maize and mCry3A in MIR604 maize. The first combined-trait product will be a breeding cross of MIR162 and *Bt*11, designated *Bt*11 x MIR162, and the second will be a breeding cross of MIR162, *Bt*11, and MIR604, designated *Bt*11 x MIR162 x MIR604. Data [have] been developed by Syngenta demonstrating that issuance of each of these registrations will be in the public interest.

Field efficacy trials demonstrate that MIR162 maize and *Bt*11 x MIR162 maize hybrids provide improved protection against lepidopteran insect feeding damage when compared to the protection provided by conventional insecticides or *Bt*11 maize alone. This improved product efficacy is expected to translate into increased maize grain yield and quality. In a time of rising demand for maize grain, the MIR162 trait has the potential to provide United States (U.S.) agriculture with an economic benefit exceeding $371 million annually at product maturity. The introduction of the MIR162 trait in combination with *Bt*11 also has the potential to replace many conventional

insecticide applications, reduce greenhouse gas emissions, and [reduce] mycotoxin contamination of livestock feed. There will also be insect resistance management (IRM) benefits stemming from the introduction of these combined-trait hybrids. The Vip3Aa20 protein contained in MIR162 maize brings a second mode of action against *Helicoverpa zea* and *Spodoptera frugiperda*, two pests that are only suppressed by Cry1Ab. Data [have] been developed showing that *Bt*11 x MIR162 is high dose against these two pests; accordingly, a reduction from the 50% structured refuge requirement in the South is warranted. This will greatly benefit maize growers in the affected counties of the South as it will allow them to protect more of their maize acres against feeding damage from lepidopteran pests.

Adoption of *Bt*11 x MIR162 x MIR604 hybrids by growers is predicted to offer crop yield advantages and important new options for control of virtually all the major insect pests of maize, all built into a single seed product. The availability of a new product for lepidopteran and rootworm control will provide choices for growers in the marketplace, and lead to increased price competition for traits, which will benefit growers and others in the maize value chain. *Bt*11 x MIR162 x MIR604 maize also offers health and environmental safety advantages over the use of conventional insecticides, as well as insect resistance management benefits that will preserve the durability of this and other *Bt*-based products.

Collectively, the information presented in this document convincingly supports a public interest finding for registration of the plant-incorporated protectants in MIR162, *Bt*11 x MIR162, and *Bt*11 x MIR162 x MIR604 maize.

3. BPPD's Review of Syngenta's PID

Syngenta submitted several documents, which will be summarized, reviewed, and analyzed in this section of the BRAD: (1) efficacy studies conducted during 2006 (White *et al*. 2007a–g; Master Record Identification [MRID] Numbers 471530-01, 471530-02, 471530-03, 471530-04, 470531-01, 470531-02, and 470531-03), (2) a public interest document (Ward and Vlachos 2007; MRID Number [No.] 471378-19), and (3) an IRM chapter (Kurtz *et al*. 2007; MRID No. 471374-07). A complete IRM review can be found in the Insect Resistance Management chapter of this BRAD.

i. Public Interest Finding

a. Syngenta's Public Interest Findings (MRID No. 471378-19)

In this section of the BRAD, a summary of public interest findings from Syngenta's PID is provided. The human health and environmental benefits, as well as the insect resistance management benefits reported by Syngenta, are summarized in later sections of this BRAD chapter.

Presumption of Public Interest

MIR162 has the potential to displace the use of many of the Restricted Use Pesticides that are currently being used for control of lepidopteran pests of maize. Based on this consideration alone, the plant-incorporated Vip3Aa20 pesticidal protein encoded in MIR162 maize is entitled to a presumption of public interest.

Need Factors

As the price of maize grain continues to rise, the economic threshold for growers to respond to infestations of *A. ipsilon*, *H. zea*, *S. albicosta*, or *S. frugiperda* will fall. Even relatively small reductions in crop yield (<10%) will result in a significant economic loss for growers. Additionally, there is evidence that populations of *S. albicosta* are spreading eastward and will have the potential to cause greater harm in critical maize-producing states.

[C]ontrol of aboveground maize insect pests is challenging for growers. Conventional insecticide applications are costly and intensive scouting of fields is required to identify the appropriate timing for applications. Growers only have a very narrow time window during which insecticides can be applied because many of the aboveground feeding insects are shielded from contact with the insecticides by virtue of their feeding location on the plant. Planting of combined-trait hybrids containing [the] MIR162 [trait] will provide growers with a more effective means of controlling these economically significant insect pests of maize.

While it has not been possible to conduct direct side-by-side efficacy comparisons of Cry1F and *Bt*11 x MIR162 hybrids, *Bt*11 x MIR162 hybrids are expected to provide a level of broad lepidopteran control that is unsurpassed by currently available *Bt* hybrids or conventional insecticide products. For *H. zea*, in particular, *Bt*11 x MIR162 hybrids have been shown to provide excellent control that meets Environmental Protection Agency (EPA) insect resistance management criteria for "high dose," whereas Cry1F hybrids provide only "suppression" of this pest.

Although other stacked transgenic maize hybrids offering combined lepidopteran and coleopteran control are available in the U.S., direct efficacy comparisons with *Bt*11 x MIR162 x MIR604 hybrids

have not been possible. It is expected that *Bt*11 x MIR162 x MIR604 hybrids will provide unsurpassed control of target pests. Their excellent broad-lepidopteran control, particularly for *H. zea* and *S. albicosta*, can potentially result in better performance than competitor offerings.

Composition Factors

The active ingredient, Vip3Aa20, is plant-incorporated. It is safer than all currently registered conventional maize insecticide products. This characteristic of the product virtually eliminates the occupational and environmental risks currently associated with the application of chemical controls for maize insect pests. Registration of this product also provides EPA with an opportunity to reduce the manufacture, transportation, storage, and disposal of millions of pounds of hazardous chemicals annually and to eliminate the greenhouse gas emissions associated with these activities.

Usage Factors

The safety, convenience, and simplicity of planting MIR162 hybrids compared to the application of conventional insecticides, along with the opportunity to extract an economic benefit through increased crop yield, are expected to make this product attractive to growers.

Performance Factors

Two years of extensive efficacy field trials, conducted at multiple locations under varying levels of insect pressure, have demonstrated the superior leaf, stalk, and ear protection provided by MIR162 maize compared to hybrids treated with a conventional insecticide product…Furthermore, the delivery of Vip3Aa20 in the maize seed and its production in plants eliminates many risks associated with conventional insecticide usage, some of which include improper calibration and maintenance of application equipment, handling of hazardous chemical insecticides, container disposal, chemical misplacement, runoff, and spray drift.

Timing of application is not a factor with MIR162 hybrids since Vip3Aa20 is present in the plant throughout the growing season. Planting of MIR162 hybrids is compatible with current insect scouting and monitoring programs that provide data upon which to base crop management decisions. The product is also fully compatible with cultural control measures such as crop rotation. MIR162 fits seamlessly into the concept of integrated pest management (IPM) for maize. Superior protection of crop yield and a seamless fit with IPM programs indicate that registration of MIR162 maize is in the public interest.

*Bt*11 x MIR162 maize will combine the efficacy of *Bt*11 maize and MIR162 maize to provide broad-spectrum control of major U.S. lepidopteran maize pests at a level that will outperform current technologies…Collectively, the results of field efficacy trials demonstrate that *Bt*11 x MIR162 maize will be protected from feeding damage caused by the following insect pests: *O. nubilalis*, *D. grandiosella*, *Diatraea crambidoides* (southern cornstalk borer), *H. zea*, *S. frugiperda*, *Papaipema*

nebris (common stalk borer), *Diatraea saccharalis* (sugarcane borer), *A. ipsilon*, *S. albicosta*, and *Spodoptera exigua* (beet armyworm).

Combining Cry1Ab, Vip3Aa20, and mCry3A traits in a single maize hybrid retains the insect control efficacy of the individual proteins. Accompanying the present submission are reports of efficacy studies in *O. nubilalis*, *H. zea*, *S. frugiperda*, and *D. virgifera virgifera* that substantiate the predicted efficacy of combining multiple insecticidal traits in *Bt*11 x MIR162 x MIR604 maize hybrids. Therefore, it is reasonable to assume that growers will realize the cumulative benefits of all three insecticidal traits in this product.

Risk Factors

Fusarium ear rot is the most common ear rot disease in the Midwest and is closely associated with insect feeding damage to maize ears. Although the disease does not cause significant yield loss, it reduces grain quality, and increases the fungi that can produce mycotoxins, such as fumonisins. Mycotoxin contamination of maize grain presents a potential threat to livestock health and it is occasionally necessary to reject or reformulate [feed] lots because of contamination. Due to the superior protection from insect ear feeding damage that will be afforded by planting MIR162 hybrids, there is a potential health benefit for the livestock industry resulting from reduced mycotoxin levels in livestock feed.

Thus, the introduction of MIR162 technology has the potential to reduce applications of conventional insecticides and improve grain quality by reducing mycotoxin levels. These facts indicate that registration of MIR162 is in the public interest.

An additional food and feed safety benefit of *Bt*11 x MIR162 is its potential to reduce the levels of fumonisin, a harmful fungal toxin, in maize grain. [G]rain from *Bt* maize hybrids (including *Bt*11 maize) is associated with significantly reduced levels of fumonisin. This is an indirect benefit of protecting maize ears from feeding damage by lepidopteran pests. The additional control of ear-feeding pests, particularly *H. zea* and *S. albicosta*, that will be provided by *Bt*11 x MIR162 maize will likely further reduce mycotoxin contamination in grain.

b. BPPD's Response

Syngenta claims that "[i]t is expected that *Bt*11 x MIR162 x MIR604 hybrids will provide unsurpassed control of target pests" and that the product's "broad-lepidopteran control, particularly for *H. zea* and *S. albicosta*, can potentially result in better performance than competitor offerings." BPPD notes that these statements are unverified assumptions.

BPPD concludes that MIR162 maize, *Bt*11 x MIR162 corn, and *Bt*11 x MIR162 x MIR604 corn are expected to provide the public interest benefits shared by other corn PIPs already registered by the

Agency. Specifically, stacked and pyramided *Bt*11 x MIR162 x MIR604 corn would provide a new tool for farmers who face challenges of protecting corn crops from lepidopteran as well as coleopteran pest damage. In addition, both stacked and/or pyramided products (*Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn) can be expected to prolong the lifetime of corn PIPs due to Vip3A having a novel mode of action.

A more detailed analysis of human health, environmental, and IRM benefits will follow in upcoming sections of this BRAD chapter.

ii. Efficacy Data

*Bt*11 corn plants express a truncated Cry1Ab insecticidal protein for control of certain lepidopteran pests (i.e., ECB) and a phosphinothricin acetyltransferase (PAT) protein that confers tolerance to herbicide products containing glufosinate. MIR162 maize plants express Vip3Aa20 insecticidal protein to control FAW, CEW, and WBCW (and other lepidopteran pests) and a phosphomannose isomerase (PMI) protein that acts as a selectable marker trait enabling transformed plant cells to utilize mannose as a primary carbon source. MIR604 corn plants express modified Cry3Aa insecticidal protein for control of certain coleopteran pests (i.e., WCR and NCR) and a similar PMI protein as a selectable marker. GA21 maize plants express a double-mutated 5-enol pyruvylshikimate-3-phosphate synthase (mEPSPS) protein that confers a tolerance to herbicide products containing glyphosate.

Below are BPPD's summaries of Syngenta's efficacy studies from the 2006 corn-growing season (MRID Numbers 471530-01, 471530-02, 471530-03, 471530-04, 471531-01, 471531-02, and 471531-03).

a. Efficacy of Bt11 x MIR162 x MIR604 x GA21 Against ECB (MRID No. 471530-01)

The objective of the study was to test whether ECB control efficacy by *Bt*11 corn plants is unaffected by the presence of MIR162, MIR604, and GA21 or absence of these transgenic traits. The experiment was conducted as a randomized complete block design with three replicates (10 plants each; n = 30) in Minnesota and Illinois. The four treatments were *Bt*11 x MIR162 x MIR604 x GA21 corn, MIR162 maize, *Bt*11 corn, and a non-transgenic hybrid. Two artificial infestations (simulating 2 generations of ECB in the field) were conducted with laboratory-reared neonates at a rate of 150 larvae per plant during the first application (at whorl stage) and 200 larvae per plant during the second application (at pollen shed stage). Foliar leaf damage was assessed using the Guthrie scale of 1–9 (see Table 1 below) for 10 consecutive plants in a row, 14 days after infestation. Forty-five days after the second infestation, ten consecutive plants were dissected to assess ear shank, ear kernel, and stalk feeding by measuring feeding tunnel lengths. Both types of data collected were analyzed using analysis of variance (ANOVA).

No significant difference ($p \leq 0.05$) in ECB efficacy (foliar leaf damage; ear shank and stalk feeding damage) was observed between *Bt*11 corn and *Bt*11 x MIR162 x MIR604 x GA21 corn plants when the data from both locations were pooled and analyzed separately. There was one occasion of significant difference in second-generation ECB ear feeding damage at the Illinois location. However, this significant difference disappeared when data were pooled. Syngenta did not provide an explanation as to why there might have been a significant difference at the Illinois location. Significant differences were observed between *Bt*11-containing hybrid plants and MIR604 and control plants when data were separated by location or pooled (foliar leaf damage; ear, ear shank, and stalk feeding damage). *Bt*11-containing hybrid plants provided excellent protection against ECB, while damage to MIR604 and control plants was much higher.

Table 1. Leaf Damage Rating Scale for ECB (Guthrie *et al.* 1960)

First Generation ECB Rating Class	Description of Damage
1	No damage or damage limited to a few spots no larger than a pinprick (≤ 0.5 millimeter [mm] in diameter).
2	Tiny holes all ≤ 2 mm and only on one or two leaves. Many not chewed through leaf.
3	Small shot-hole feeding scars on several (approximately 3+) leaves; a few may be slightly larger than 2 mm in diameter but still round.
4	Holes on several leaves are somewhat square or irregularly shaped but length is less than 3x the width of hole (≤ ½ inch long).
5	Elongate lesions at least 3x as long as wide (approximately ¾ inch–1 inch long) on 1–3 leaves.
6	Lesions on several (approximately 3+) leaves are ≥ 1 inch long (2.54 centimeters).
7	Long lesions (1 inch or longer) common on ½ of leaves, and with some lesions merging together from the sides or ends.
8	Many long lesions merging; merging common on about ½ of leaves; 1–2 leaves on plant appear shredded; midrib boring.
9	Most leaves with long and merging lesions; plant has a shredded appearance with substantial midrib breakage usually.

b. Efficacy of Bt11 x MIR162 x MIR604 x GA21 Against CEW (MRID No. 471530-02)

The objective of the study was to test the hypothesis that CEW control efficacy by MIR162 maize plants is unaffected by the presence of *Bt*11, MIR604, and GA21 or absence of these transgenic traits. The experiment was conducted as a randomized complete block design with three replicates in Iowa (five plants per replicate; n = 15) and Illinois (six plants per replicate; n = 18). Artificial infestations were conducted with laboratory-reared neonates and approximately 20 larvae per plant were applied to green silks of the most developed ear on each plant. Ear feeding damage was

assessed using the modified Widstrom scale (see Table 2 below) 14 days after infestation. Data collected were analyzed using ANOVA.

There was a significant difference in ear feeding damage between *Bt*11 x MIR162 x MIR604 x GA21 corn plants and MIR162 maize plants as compared to *Bt*11 corn plants, indicating that the MIR162 trait provides excellent protection against CEW damage. While there was no statistically significant difference between the two hybrids containing the MIR162 trait, ear feeding damage was numerically lower on *Bt*11 x MIR162 x MIR604 x GA21 corn plants than on MIR162 maize plants alone, suggesting the *Bt*11 trait provides some protection against CEW. Damage on control plants was statistically significantly different from damage caused on *Bt*11 corn plants supporting that the *Bt*11 trait provides some protection from CEW damage.

Table 2. Ear Feeding Damage Rating for CEW (Widstrom 1967)

Rate	Description
0	No damage to silks, husks, cob tip or kernels
1	Light to moderate damage to silks and cob but not husk or kernel damage
2	Damage to silks and cob but little husk damage with 0.1–1.0 centimeter (cm) of kernel damage/kernel loss
3	1.1–2.0 cm of kernel damage/loss
4	2.1–3.0 cm of kernel damage/loss
5	3.1–4.0 cm of kernel damage/loss
6	4.1–5.0 cm of kernel damage/loss
7	6 cm of kernel damage/loss
8	7 cm of kernel damage/loss
9	8 cm of kernel damage/loss
10	cm of kernel damage/loss +1

c. Efficacy of Bt11 x MIR162 x MIR604 x GA21 Against FAW (MRID No. 471530-03)

The objective of the study was to test that FAW control efficacy by MIR162 maize plants is unaffected by the presence of *Bt*11, MIR604, and GA21 or absence of these transgenic traits. The experiment was conducted as a randomized complete block design with three replicates (10 plants each; n = 30) in Minnesota and Illinois. Two artificial infestations were conducted with laboratory-reared neonates and approximately 80 larvae per plant were placed into the whorl of each plant.

Foliar feeding damage was assessed 14 days after infestation using a modified version of the Davis scale (see Table 3 below). Data collected were analyzed using ANOVA.

Plants containing either the MIR162 trait or the MIR162 trait combined with other traits suffered slight damage from FAW larvae and damage ratings differed significantly from *Bt*11 corn ratings. FAW were destructive to non-transgenic corn plants, and those damage ratings differed significantly from all other treatments. The results confirm that plants containing the MIR162 insecticidal trait alone or in a stack and pyramid as *Bt*11 x MIR162 x MIR604 x GA21 provide excellent control against FAW.

Table 3. Modified Davis Scale for Foliar Feeding Damage Ratings by FAW (Davis *et al.* 1992)

Rating	Description
1	No visible damage or only pinhole lesions present on whorl leaves.
2	Pinhole and small circular lesions present on whorl leaves.
3	Small circular lesions and a few small elongated (rectangular shaped) lesions of up to 1.3 cm in length present on whorl and furl leaves.
4	Several small to mid-sized 1.3 to 2.5 cm in length elongated lesions present on a few whorl and furl leaves.
5	Several large elongated lesions greater than 2.5 cm in length present on a few whorl and furl leaves and/or a few small to mid-sized uniform to irregular shaped holes (basement membrane consumed) eaten from the whorl and/or furl leaves.
6	Several large elongated lesions present on several whorl and furl leaves and/or several large uniform to irregular-shaped holes eaten from furl/whorl leaves.
7	Many elongated lesions of all sizes present on several whorl and furl leaves and/or several large uniform to irregular-shaped holes eaten from furl/whorl leaves.
8	Many elongated lesions of all sizes present on most whorl and furl leaves plus many mid- to large-sized uniform to irregular-shaped holes eaten from the whorl and furl leaves.
9	Whorl and furl leaves almost totally destroyed. Many elongated lesions of all sizes.

d. Efficacy of Bt11 x MIR162 x MIR604 x GA21 Against WCR (MRID No. 471530-04)

The objective of the study was to test that CRW control efficacy by MIR604 corn plants is unaffected by the presence of *Bt*11, MIR162, and GA21 or absence of these transgenic traits. The experiment was conducted as a randomized complete block design with three replicates (6 plants

each; n = 18) in Minnesota and Illinois. The three treatments were *Bt*11 x MIR162 x MIR604 x GA21 corn, MIR604 corn, and a non-transgenic hybrid. One artificial infestation was conducted with WCR eggs at a rate of 1,500 eggs per plant at V2 (second leaf)–V3 (third leaf) stage of plant development. In Illinois, the trial was conducted in a field that had been planted to a trap crop for WCR the previous season to attract beetles for increased egg accumulation. Damage ratings (see Table 4 below) were taken on roots collected and washed just prior to the silk stage. Six root samples per plot were selected at both locations. ANOVA was used to analyze the data.

Plants containing the MIR604 trait had significantly less damage than control plants. No statistical difference was detected between MIR604 plants and *Bt*11 x MIR162 x MIR604 x GA21 plants.

Table 4. Linear Root Feeding Rating Scale for WCR (Adapted from Oleson 2005)

Rating	Description of Rootworm Damage
0.01	No damage to 1–2 light surface scars on roots
0.02	3+ light surface scars ≤ 4 moderate scars (combined across all roots on a plant)
0.05	5+ heavy scars (long, deep scars) but NO root pruning (pruning ≤ 1.5 inches from crown)
0.10	One root pruned to ≤ 1.5 inches accompanied by heavy scars
0.25	2+ roots pruned to ≤ 1.5 inches (up to ¼ nodes, equivalent, pruned)
0.50	Equivalent of 0.50 node of roots pruned
0.75	Equivalent of 0.75 node of roots pruned
1.00	Equivalent of 1.00 node of roots pruned
1.25	Equivalent of 1.25 node of roots pruned
1.50	Equivalent of 1.50 node of roots pruned
1.75	Equivalent of 1.75 node of roots pruned
2.00	Equivalent of 2.00 node of roots pruned
2.25	Equivalent of 2.25 node of roots pruned
2.50	Equivalent of 2.50 node of roots pruned
2.75	Equivalent of 2.75 node of roots pruned
3.00	Equivalent of 3.00 node of roots pruned

e. Efficacy of Bt11 x MIR162 x GA21 Against ECB (MRID No. 471531-01)

The objective of the study was to compare the ECB control efficacy by *Bt*11 x MIR162 x GA21 corn plants to the efficacy of hybrids containing only the MIR162 or *Bt*11 traits. Study methodology was

identical to the ECB study mentioned under section II(E)(3)(ii)(a) of this chapter.

No significant difference ($p \leq 0.05$) in ECB efficacy (foliar leaf damage) was observed between *Bt*11 corn and *Bt*11 x MIR162 x GA21 corn when the data from both locations were analyzed separately or pooled. Both hybrids provided excellent protection against first-generation ECB. Significant differences were observed between *Bt*11-containing hybrid plants and MIR162 and control plants when data were separated by location or pooled. Second-generation ECB damage to MIR162 and control plants was significantly higher than to *Bt*11 x MIR162 x GA21 corn plants. Both hybrids containing the *Bt*11 trait provided excellent protection against ECB. There was a small yet statistically significant level of suppression at one location (Illinois) by MIR162 maize plants against ECB ear and stalk feeding when compared to control plants.

f. Efficacy of Bt11 x MIR162 x GA21 Against CEW (MRID No. 471531-02)

The objective of the study was to compare the CEW control efficacy by *Bt*11 x MIR162 x GA21 corn plants to the efficacy of hybrids containing only the MIR162 or *Bt*11 traits. Study methodology was identical to the CEW study mentioned under section II(E)(3)(ii)(b) of this chapter.

CEW larvae caused slight damage to treatment plants containing the MIR162 event and there was no statistical difference between damage ratings of the two treatments, MIR162 maize and *Bt*11 x MIR162 x GA21 corn. There was a significant difference in ear feeding damage between the two treatments containing the MIR162 trait alone or in the stack and the *Bt*11 treatment (Illinois location). When the data were pooled from both locations, the difference disappeared. Syngenta did not provide an explanation for this result. However, BPPD noticed that mean ear rating reported for the *Bt*11 treatment was much greater than that reported for the Iowa location. Damage on control plants was statistically significantly different from damage caused on *Bt*11 corn plants supporting that the *Bt*11 trait provides some protection from CEW damage. Damage to MIR162-containing hybrids was slight.

g. Efficacy of Bt11 x MIR162 x GA21 Against FAW (MRID No. 471531-03)

The objective of the study was to test that FAW control efficacy by MIR162 maize plants is unaffected by the presence or absence of the *Bt*11 trait. Study methodology was identical to the FAW study mentioned under section II(E)(3)(ii)(c) of this chapter.

Both treatment plants containing MIR162 alone or stacked with *Bt*11 and GA21 provided excellent control against FAW, and no significant difference between the two damage ratings was observed. The *Bt*11 treatment plants suffered greater damage and their damage rating differed significantly from the two MIR162 treatment ratings. The control plants suffered the greatest damage, and their damage rating differed from all other treatments.

h. BPPD's Response

Matten (2007) reviewed the results of a small-scale field trial conducted at multiple locations during the 2005 corn-growing season. One of the comments in that review was that the MIR604 trait, when combined with the MIR162 trait, showed some evidence of a possible synergistic effect in the control of corn rootworm. In Syngenta's newest efficacy study reviewed above (2006 growing season), the sample sizes chosen per treatment are extremely small ($N_{WCR} = 6$, 5 and $N_{WCR} = 18$, 15). The power to detect significant differences between treatments of WCR depends heavily on the sample size chosen. BPPD is concerned that Syngenta may not have a basis for testing their hypothesis that "the control of corn rootworm by hybrids containing the MIR604 trait is the same regardless of the presence of other transgenic traits" or whether there are synergistic effects when the MIR604 trait is combined with the MIR162 trait. Specifically, BPPD expects the difference in WCR damage to be smaller between MIR604 corn plants and *Bt*11 x MIR162 x MIR604 x GA21 corn plants than between MIR604 corn plants and control plants and has doubts that Syngenta's experiments have enough power to detect these smaller differences between treatments due to the very small sample size chosen. BPPD would like to know what the rationale was for choosing such small samples.

BPPD concludes the following from the review of the 2006 efficacy studies:

- There are indications from the Illinois data (ECB and CEW efficacy studies) that stacking the *Bt*11, MIR162, MIR604, and GA21 traits together may produce different efficacy results than for *Bt*11 corn plants alone. Whether this is due to synergistic effects between the toxins or environmental effects is unclear, and Syngenta did not provide an explanation for the results. In future submissions of efficacy studies, BPPD requests that Syngenta address such differences by supplying possible explanations. However, BPPD notes that both the stacked and/or pyramided and single-trait products appear to provide good protection against ECB and CEW.

- The data support that the stack and pyramid containing the *Bt*11, MIR162, MIR604 and GA21 traits provides good efficacy results against FAW.

- The data support that the stack and pyramid containing the *Bt*11, MIR162, MIR604 and GA21 traits produces reasonably good efficacy against WCR.

- No definitive conclusion can be reached regarding synergistic effects because the sample sizes chosen appear to be too small.

iii. Grower Benefits

a. Summary of Syngenta's Submission in the PID (MRID No. 471378-19)

At the request of Syngenta, a study was undertaken by agricultural economists at North Carolina State University to develop an estimate of the value to U.S. farmers of the MIR162 maize trait technology. First, they considered the potential economic effects of MIR162 introduction on the market for existing insect-protection trait technologies. Second, they commissioned a grower survey to assess willingness to adopt the new technology. Lastly, they estimated the spatial distribution of the costs of control for *H. zea* and *S. albicosta* and how these costs might change in future years. [A shortened summary of this report follows below.]

Following general economic principles, the introduction of a new technology will have an effect on the market for existing technologies that is beneficial to users of either technology. This will come in the form of downward pressure on prices of the competing technologies. This is beneficial to growers because prices of maize traits will tend to remain lower and more stable in the future than would otherwise be the case.

From data collected in a telephone survey of 150 maize growers in 12 states, average yield losses in 2006 attributable to *H. zea* were estimated to be 4.9 bushels/acre (bu/ac) and losses attributable to *S. albicosta* were estimated to be 4.8 bu/ac. Examination of data provided by these growers for the past five seasons suggests that yield losses attributable to the two pests are increasing. This conclusion is supported by analysis of insecticide use data for 2005 and 2006, which indicate that economically significant infestations of *H. zea* and *S. albicosta* are on the rise in the Corn Belt and Great Plains. As the price of maize grain increases, the amount of feeding damage needed to exceed an economic threshold for applying corrective measures decreases. The grower survey results indicate that 70% of respondents would purchase MIR162 hybrids if they were available and would plant them on an average of 500 acres per farm four years after introduction.

A potential economic benefit for maize growers from the commercial introduction of MIR162 hybrids has been computed in the form of an upper-bound estimate for the three largest maize-producing states (Iowa, Illinois, and Nebraska)…Providing growers with a means to effectively control *H. zea* and *S. albicosta* in these three states alone provides an economic benefit of up to $371 million annually. This is an upper-bound estimate on value available to growers; it assumes a 100% market share for MIR162 hybrids and does not take into account potential price responses for competitive products. Ultimately, some portion of the economic gain derived by growers using this new technology will be passed along to consumers in the form of lower commodity prices. These substantial economic benefits indicate that registration of MIR162 is in the public interest.

For many growers, the broad lepidopteran control offered by *Bt*11 x MIR162 hybrids will represent a higher insurance value than currently available *Bt* products. Additionally, *Bt*11 x MIR162 hybrids will offer unsurpassed convenience to growers by reducing the need to scout fields for pest pressure or to apply other control measures for lepidopteran larvae.

The broad efficacy of *Bt*11 x MIR162 x MIR604 hybrids will provide "insurance" for growers against damage by multiple pests that might otherwise cause significant economic loss in any given year. The same broad efficacy will provide convenience for growers, as they will be able to eliminate the need to apply both a soil insecticide for control of *Diabrotica* rootworms and *A. ipsilon*, and a foliar insecticide later in the season for foliar insects. It will also reduce or eliminate their need to scout fields for pest pressure.

Commodity prices for maize grain have dramatically increased recently due, [in part], to high demand for fuel ethanol, and sustained demand is predicted for the coming years. Such demand will operate to increase the value of a grower's investment in any agricultural practice, technology, or maize traits that increase or preserve yield.

Another predicted economic benefit for growers and downstream consumers is increased competition in the marketplace for pest-control products, including hybrid seed from multiple providers of lepidopteran-active and/or rootworm-active transgenic varieties. The commercial availability of *Bt*11 x MIR162 x MIR604 hybrid maize seed will represent a significant new pest control option and tool available to growers. Increased grower choice can be expected to exert downward pressure on the cost of products that offer control of lepidopteran and rootworm pests.

b. BPPD's Response

BPPD focuses on the benefits from MIR162 maize, *Bt*11 x MIR162 corn, and *Bt*11 x MIR162 x MIR604 corn only and not on the potential economic benefits of these products containing the GA21 trait. It may be that additional benefits are derived from an herbicide-tolerance trait in MIR162 maize, *Bt*11 x MIR162 corn, and *Bt*11 x MIR162 x MIR604 corn. On the other hand, such a trait could also increase the risk of weed resistance.

BPPD finds that MIR162 maize, *Bt*11 x MIR162 corn, and *Bt*11 x MIR162 x MIR604 corn will likely have similar economic grower benefits of already registered corn PIPs (i.e., *Bt*11 and MIR604) as described by the Agency in the 2001 *Bt* Crops Reassessment and MIR604 BRAD. The Agency's summary of these benefits can be accessed online at *http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm* and *http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006509.pdf*. In addition, *Bt*11 x MIR162 x MIR604 corn will provide further benefits by controlling corn rootworm as well as several lepidopteran pests.

Syngenta's specific economic benefits are based on best-case assumptions (i.e., quick and broad adoption of the product in the marketplace). Competition from previously registered *Bt* corn products (already established in the market) may reduce the overall benefits for MIR162 maize and its associated products. Nevertheless, growers planting MIR162 maize (and its stacked and/or pyramided products) will realize significant economic benefits, particularly growers with multiple pest problems.

iv. Human Health and Environmental Benefits

a. Summary of Syngenta's Submission in the PID (MRID No. 471378-19)

A standard battery of mammalian toxicity studies failed to provide any evidence of Vip3Aa20-induced adverse effects. The protein is rapidly degraded in mammalian digestive systems and it bears no [significant] amino acid sequence similarities to known toxins and allergens. Since the insecticidal protein is plant-incorporated, the opportunity for exposure when handling and planting seed is minimal. Planting of MIR162 hybrids will essentially eliminate the occupational health risks currently associated with chemical controls for leaf- and ear-feeding insect pests.

The selectivity of Vip3Aa20 for lepidopteran pests minimizes risk for non-target insects. A series of hazard identification studies has been conducted with non-target indicator species, including many species that are part of the maize ecosystem. No adverse effects attributable to Vip3Aa proteins were observed in these studies, even at exposure levels exceeding expected environmental concentrations.

The combined mammalian and environmental safety profile of *Bt*11 x MIRl62 indicates that the product will pose no significant risks. Accordingly, it offers health and environmental advantages over current chemical alternatives for control of lepidopteran pests.

The combined mammalian and environmental safety profile of *Bt*11 x MIR162 x MIR604 maize indicates that the product will pose no significant safety risks. Accordingly, it offers significant health and environmental advantages over current chemical alternatives for control of lepidopteran and rootworm pests. For maize growers who currently rely upon conventional insecticide applications for lepidopteran and rootworm control, *Bt*11 x MIR162 x MIR604 maize will allow them to significantly reduce, if not eliminate, the need to apply chemical controls for these pests. This will represent both a reduced health and safety risk for agricultural workers and will reduce the impact of insecticide use on wildlife and the environment.

b. BPPD's Response

EPA reviewed product characterization, human health safety, and aquatic and terrestrial wildlife studies submitted by Syngenta and agrees with Syngenta's conclusions. There is no human health concern with respect to toxicity or allergenicity and no environmental concern with respect to

toxicity of the insecticidal proteins expressed in MIR162 maize(i.e., Vip3Aa), *Bt*11 x MIR162 corn (i.e., Cry1Ab andVip3Aa), or *Bt*11 x MIR162 x MIR604 corn (i.e., Cry1Ab, Vip3Aa, and mCry3A). For information regarding the Agency's conclusion on *Bt*11 benefits with respect to human health and environment, the 2001 *Bt* Crops Reassessment (*http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm*) and the Biopesticides Registration Action Document for the modified Cry3A protein (*http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006509.pdf*) can be consulted.

v. Insect Resistance Management Benefits

a. Summary of Syngenta's Information in the PID (MRID No. 471378-19) and IRM Submission (MRID No. 471374-07)

Use of *Bt*11 x MIRl62 maize offers…insect resistance management benefits that will help to preserve the durability of this and other *Bt*-based products for lepidopteran control…[T]he CrylAb and Vip3Aa20 proteins are present in these hybrids at levels that have been demonstrated to provide a high-dose for control of *O. nubilalis*, *H. zea*, and *S. frugiperda*, thus minimizing the risk of resistance developing in these species. *Bt*11 x MIR162 hybrids offer IRM advantages in comparison to other control options that do not demonstrably provide a "high dose" against the target pests. Moreover, Vip3Aa20 operates by a mode of action different from that of CrylAb or CrylF and targets a unique binding site(s) in susceptible larvae. The available data support a conclusion that Vip3Aa20 shows no potential for cross-resistance with Cry proteins. Thus, for *H. zea* and *S. frugiperda*, which are sensitive to both CrylAb and Vip3Aa20, *Bt*11 x MIR162 maize is predicted to significantly extend the durability of both traits for control of these pests because local populations are very unlikely to evolve resistance to two proteins that act on independent target sites.

The possibility of resistance development in *H. zea* has been of particular concern to the EPA, as it is also a pest of cotton and has the potential to undergo selection pressure from both *Bt* maize and *Bt* cotton varieties that express similar Cry proteins, where the two crops are grown in the same geographies. The principal reason that the EPA requires growers in cotton-growing areas to plant 50% of their maize acres to non-*Bt* maize hybrids concerns the potential for resistance evolution in *H. zea* populations…Syngenta provides data and rationale to justify reduction of the maize refuge in cotton-growing areas from 50% to 20% of maize acres for growers of *Bt*11 x MIR162 maize. No other *Bt* product offers comparable IRM advantages in maize.

For growers of *Bt*11 x MIR162 maize hybrids, the reduced refuge requirement in cotton-growing regions will translate into a higher proportion of insect-protected maize acres, with a proportional increase in all the attendant benefits of the product in these areas. As an added advantage, compliance with the refuge requirement for IRM can be predicted to increase because *Bt* maize growers in cotton-growing regions have heretofore not been able to fully experience the benefits

enjoyed by *Bt* maize growers in other regions of the U.S. The potential for increased maize acres in cotton-growing regions can also help meet the current high demand for maize grain.

The same insect resistance management benefits…for *Bt*11 x MIR162 maize will also apply to *Bt*11 x MIR162 x MIR604 maize. Accordingly, a 20% non-*Bt* maize refuge in cotton-growing regions will be justified. The stacking of three insecticidal proteins in this product is not expected to increase selection pressure for cross-resistance among local pest populations, owing to the different modes-of-action and target sites for the CrylAb, Vip3Aa20, and mCry3A proteins. Because the mCry3A trait in *Bt*11 x MIR162 x MIR604 maize has good efficacy against its target rootworm pests, introduction of this product is expected to help extend the durability of other commercially available rootworm-protected *Bt* maize products.

b. BPPD's Response

BPPD has responded to the refuge reduction request separately in the Insect Resistance Management chapter of this BRAD. Since a 20% non-*Bt* corn refuge for *Bt*11 x MIR162 corn and Bt11 x MIR162 x MIR604 corn in the southern cotton-growing areas would be sufficient to manage the risk of resistance evolution to *Bt* corn and *Bt* cotton products, growers in the affected areas would likely realize some economic benefits.

Furthermore, BPPD concludes that MIR162 maize, *Bt*11 x MIR162 corn, and *Bt*11 x MIR162 x MIR604 corn have the following insect resistance management benefits: (1) high dose against FAW, ≥ "near high dose" against CEW, and ≥ "near high dose" against ECB; (2) low probability of cross-resistance developing between Vip3A and Cry1Ab/c and Vip3A and Cry2Ab as shown in *Heliothis virescens* (tobacco budworm, TBW) and CEW; and (3) potential to delay development of resistance in other corn varieties expressing Cry toxins. The introduction of MIR162 maize and its stacks and/or pyramids may have an additional benefit of prolonging the lifetime of other corn PIP technologies by providing another mode of action for ECB, CEW, FAW, and WCR. Generally, the greater the modes of action (i.e., toxin mosaic) in the landscape, the less likely resistance will develop to any one toxin.

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III. Terms and Conditions of the Registrations

1. MIR162 Maize

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:

1) The subject registration will automatically expire at midnight on December 31, 2011.

2) 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).

3) 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.

4) 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.

5) 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.

6) 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 [OPPTS] 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	November 1, 2009

Study Type	Required Data	Due Date
	for validation of such analytical method within six months from the date that the Agency requests them.	
Aquatic Invertebrate Toxicity (OPPTS 885.4240)	A 7–14 day *Daphnia* study as per the OPPTS 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 *Daphnia* 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

2. *Bt*11 x MIR162 Corn

The following terms and/or conditions are required for the FIFRA section 3(c)(7)(C) registration of *Bt*11 x MIR162 corn:

1) The subject registration will automatically expire at midnight on December 31, 2011.

2) The subject registration will be limited to Cry1Ab (*Bacillus thuringiensis* Cry1Ab delta-endotoxin protein and the genetic material necessary for its production [*via* elements of vector pZO1502] in Event *Bt*11 corn [OECD Unique Identifier: SYN-BTØ11-1]) x Vip3Aa20 (*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]) for use in field corn.

3) 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.

4) Syngenta will submit/cite all data required to support the individual plant-incorporated protectants in YieldGard® Insect Resistant Corn and MIR162 maize within the timeframes required by the terms and conditions of EPA Registration Numbers 67979-1 and 67979-14, respectively:

Study Type **Registration	Required Data	Due Date
Residue Analytical Method – Plants (OPPTS 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	November 1, 2009

Study Type **Registration	Required Data	Due Date
**MIR162 maize	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 provide to the 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.	
Aquatic Invertebrate Toxicity (OPPTS 885.4240) **MIR162 maize	A 7–14 day *Daphnia* study as per the OPPTS 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 *Daphnia* study.	November 1, 2009
Insect Resistance Management – Annual Reporting **MIR162 maize	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

5) Syngenta must submit the following data and/or information in the time frames listed:

Study Type	Required Data	Due Date
Insect Resistance Management – Dose	Because of the potential for synergistic interactions between plant-incorporated protectants in a stacked product, field efficacy studies and/or a protein expression report for Southwestern corn borer (SWCB), which show that *Bt*11 x MIR162 corn has the same dose profile as its single trait products, must be submitted as confirmatory data.	March 1, 2010
Insect Resistance Management – Grower Agreement	A copy of the grower agreement, associated stewardship documents, and written description of a system, which assures that growers will sign grower agreements and persons purchasing *Bt*11 x MIR162 corn will annually affirm that they are contractually bound to comply with the requirements of the insect resistance management (IRM) program, must be submitted.	Within 90 days of the date of registration
Insect Resistance Management – Compliance Monitoring Program	A compliance assurance program (CAP) for *Bt*11 x MIR162 corn must be submitted and must include a "phased compliance approach" that outlines instances of non-compliance to the IRM requirements and options of responding to non-compliant growers. This compliance assurance program should be harmonized with compliance assurance programs already in place for previously registered Syngenta *Bt* corn products.	Within 90 days of the date of registration
Insect Resistance Management – Resistance Monitoring	Baseline susceptibility and diagnostic concentration determinations for SWCB and corn earworm (CEW) to Vip3Aa20 must be submitted.	August 31, 2010

Study Type	Required Data	Due Date
Insect Resistance Management – Resistance Monitoring	A detailed Vip3Aa20 resistance monitoring plan, integrating standard procedures developed by the Agricultural Biotechnology Stewardship Technical Committee (ABSTC) and similar in structure to those established for previously registered Syngenta *Bt* corn products, for the key target pests of CEW and SWCB must be submitted.	Within 90 days of the date of registration
Insect Resistance Management – Remedial Action Plan	A final remedial action plan for the Vip3Aa20 toxin expressed in *Bt*11 x MIR162 corn, integrating the standard procedures developed by the ABSTC and harmonized with remedial action plans established for previously registered Syngenta *Bt* corn products, must be submitted.	Within 90 days of the date of registration

6) The insect resistance management terms and conditions for *Bt*11 x MIR162 corn are as follows.

The required IRM program for *Bt*11 x MIR162 corn must have the following elements:

- Requirements relating to creation of a non-*Bt* corn and/or non-lepidopteran-resistant *Bt* corn refuge in conjunction with the planting of any acreage of *Bt*11 x MIR162 corn;
- Requirements for Syngenta to prepare and require *Bt*11 x MIR162 corn users to sign "grower agreements," which impose binding contractual obligations on the grower to comply with the refuge requirements;
- Requirements regarding programs to educate growers about IRM requirements;
- Requirements regarding programs to evaluate and promote growers' compliance with IRM requirements;
- Requirements regarding programs to evaluate whether there are statistically significant and biologically relevant changes in target insect susceptibility to Vip3Aa20 and/or Cry1Ab proteins in the target insects;
- Requirements regarding a "remedial action plan," which contains measures Syngenta would take in the event that any field-relevant insect resistance was detected as well as to report on activity under the plan to EPA;
- Annual reports on units sold by state (units sold by county level will be made available to the Agency upon request), IRM grower agreements results, and the compliance assurance program including the educational program on or before January 31st each year, beginning in 2010.

a) Refuge Requirements for *Bt*11 x MIR162 Field Corn

These refuge requirements do not apply to seed increase/propagation of inbred and hybrid seed corn up to a total of 20,000 acres per county and up to a combined U.S. total of 250,000 acres per plant-incorporated protectant (PIP) active ingredient per registrant per year.

Grower agreements (also known as stewardship agreements) will specify that growers must adhere to the refuge requirements as described in the grower guide/product use guide and/or in supplements to the grower guide/product use guide.

- Specifically, growers must plant a structured refuge of at least 20% non-*Bt* corn and/or non-lepidopteran-resistant *Bt* corn that may be treated with insecticides, as detailed below, to control lepidopteran stalk-boring and other pests.
- Refuge planting options include: separate fields, blocks within fields (e.g., along the edges or headlands), perimeter strips, and strips across the field.
- External refuges must be planted within ½ mile.
- When planting the refuge as strips across the field or as perimeter strips, refuges must be at least four consecutive rows wide.
- Insecticide treatments for control of European corn borer (ECB), CEW, SWCB, and other lepidopteran pests listed on the label, grower guides, or other educational material may be applied only if economic thresholds are reached for one or more of these target pests. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents or crop consultants). Instructions to growers will specify that microbial *Bt* insecticides must not be applied to non-*Bt* corn and/or non-lepidopteran resistant *Bt* corn refuges.

b) Grower Agreement for *Bt*11 x MIR162 Corn

1) Persons purchasing *Bt*11 x MIR162 corn must sign a grower agreement. The term "grower agreement" refers to any grower purchase contract, license agreement, or similar legal document.

2) The grower agreement and/or specific stewardship documents referenced in the grower agreement must clearly set forth the terms of the current IRM program. By signing the grower agreement, a grower must be contractually bound to comply with the requirements of the IRM program.

3) Syngenta must implement a system (equivalent to what is already approved for previously registered Syngenta *Bt* corn products), which is reasonably likely to assure that persons purchasing *Bt*11 x MIR162 corn will affirm annually that they are contractually bound to comply with the requirements of the IRM program. A description of the system must be submitted to EPA within 90 days from the date of registration.

4) Syngenta must use an approved grower agreement and must submit to EPA, within 90 days from the date of registration, a copy of that agreement and any specific stewardship documents referenced in the grower agreement. If Syngenta wishes to change any part of

the grower agreement or any specific stewardship documents referenced in the grower agreement that would affect either the content of the IRM program or the legal enforceability of the provisions of the agreement relating to the IRM program, thirty days prior to implementing a proposed change, Syngenta must submit to EPA the text of such changes to ensure that it is consistent with the terms and conditions of this registration.

5) Syngenta must implement an approved system (equivalent to what is already approved for previously registered Syngenta *Bt* corn products), which is reasonably likely to assure that persons purchasing *Bt*11 x MIR162 corn sign grower agreement(s). A description of the system must be submitted to EPA within 90 days from the date of registration.

6) Syngenta shall maintain records of all *Bt*11 x MIR162 corn grower agreements for a period of three years from December 31st of the year in which the agreement was signed.

7) Beginning on January 31, 2010 and annually thereafter, Syngenta shall provide EPA with a report on the number of units of *Bt*11 x MIR162 corn seed shipped and not returned, and the number of such units that were sold to persons who have signed grower agreements. The report shall cover the time frame of a twelve-month period. Note: The first report shall contain the specified information from the time frame starting with the date of registration and extending through the 2009 growing season.

8) Syngenta must allow a review of the grower agreements and grower agreement records by EPA or by a State pesticide regulatory agency if the State agency can demonstrate that confidential business information, including names, personal information, and grower license number, will be protected.

c) IRM Education and IRM Compliance Monitoring Program for *Bt*11 x MIR162 Corn

1) Syngenta must design and implement a comprehensive, ongoing IRM education program designed to convey to *Bt*11 x MIR162 corn users the importance of complying with the IRM program. The education program shall involve the use of multiple media, e.g. face-to-face meetings, mailing written materials, EPA-reviewed language on IRM requirements on the bag or bag tag, and electronic communications such as by internet, radio, or television commercials. Copies of the materials will be provided to EPA for their records. The program shall involve at least one written communication annually to each *Bt*11 x MIR162 corn user separate from the grower technical guide. The communication shall inform the user of the current IRM requirements. Syngenta shall coordinate its education program with the educational efforts of other registrants and other organizations, such as the National Corn Growers Association and state extension programs.

2) Annually, Syngenta shall revise, and expand as necessary, its education program to take into account the information collected through the compliance survey required under paragraph 6 and from other sources. The changes shall address aspects of grower compliance that are not sufficiently high.

3) Beginning January 31, 2010, Syngenta must provide a report to EPA summarizing the activities it carried out under its education program for the prior year. Annually thereafter, Syngenta must provide EPA any substantive changes to its grower education activities as part of the overall IRM compliance assurance program report. Syngenta must either submit a separate report or contribute to the report from the industry working group, ABSTC.

4) Syngenta must design and implement an ongoing IRM compliance assurance program designed to evaluate the extent to which growers purchasing *Bt*11 x MIR162 corn are complying with the IRM program and that takes such actions as are reasonably needed to assure that growers who have not complied with the program either do so in the future or lose their access to *Bt*11 x MIR162 corn. Syngenta shall coordinate with other *Bt* corn registrants in designing and implementing its compliance assurance program and integrate this registration into the current compliance assurance program used for their other *Bt* corn PIPs. Syngenta must prepare and submit within 90 days of the date of registration a written description of the compliance assurance program. Other required features of the program are described in paragraphs 5–15 below.

5) Syngenta must establish and publicize a "phased compliance approach," i.e., a guidance document that indicates how they will address instances of non-compliance with the terms of the IRM program and general criteria for choosing among options for responding to any non-compliant growers. While recognizing that for reasons of difference in business practices there are needs for flexibility between different companies, Syngenta must use a consistent set of standards for responding to non-compliance. The options shall include withdrawal of the right to purchase *Bt*11 x MIR162 corn for an individual grower or for all growers in a specific region. An individual grower found to be significantly out of compliance two years in a row would be denied sales of *Bt*11 x MIR162 corn the next year. Similarly, seed dealers who are not fulfilling their obligations to inform/educate growers of their IRM obligations will lose their opportunity to sell *Bt*11 x MIR162 corn.

6) The IRM compliance assurance program shall include an annual survey, conducted by an independent third party, of a statistically representative sample of growers of *Bt*11 x MIR162 corn who plant the vast majority of all corn in the United States and in areas in which the selection intensity is the greatest. The survey shall consider only those growers who plant 200 or more acres of corn in the Corn Belt or who plant 100 or more acres of corn in corn-cotton growing areas. The survey shall measure the degree of compliance with the IRM program by growers in different regions of the country and consider the

potential impact of non-response. The sample size and geographical resolution may be adjusted annually, based upon input from independent marketing research firms and academic scientists, to allow analysis of compliance behavior within regions or between regions. The sample size must provide a reasonable sensitivity for comparing results across the United States.

7) The survey shall be designed to provide an understanding of any difficulties growers encounter in implementing IRM requirements. An analysis of the survey results must include the reasons, extent, and potential biological significance of any implementation deviations.

8) The survey shall be designed to obtain grower feedback on the usefulness of specific educational tools and initiatives.

9) Syngenta shall provide a final written summary of the results of the prior year's survey (together with a description of the regions, the methodology used, and the supporting data) to EPA by January 31st of each year, beginning with 2010. Syngenta shall confer with other registrants and EPA on the design and content of the survey prior to its implementation.

10) Annually, Syngenta shall revise, and expand as necessary, its compliance assurance program to take into account the information collected through the compliance survey (required under paragraphs 6 through 8) and from other sources. The changes shall address aspects of grower compliance that are not sufficiently high. Syngenta must confer with the Agency prior to adopting any changes.

11) Syngenta shall conduct an annual on-farm assessment program. Syngenta shall train its representatives who make on-farm visits with growers of *Bt*11 x MIR162 corn to perform assessments of compliance with IRM requirements. There is no minimum corn acreage size for this program. Therefore, growers will be selected for this program from across all farm sizes. In the event that any of these visits result in the identification of a grower who is not in compliance with the IRM program, Syngenta shall take appropriate action, consistent with its "phased compliance approach" to promote compliance.

12) Syngenta shall carry out a program for investigating legitimate "tips and complaints" that its growers are not in compliance with the IRM program. Whenever an investigation results in the identification of a grower who is not in compliance with the IRM program, Syngenta shall take appropriate action, consistent with its "phased compliance approach."

13) If a grower, who purchases *Bt*11 x MIR162 corn for planting, was specifically identified as not being in compliance during the previous year, Syngenta shall visit with the grower and evaluate whether that the grower is in compliance with the IRM program for the

current year.

14) Beginning January 31, 2010 and annually thereafter, Syngenta shall provide a report to EPA summarizing the activities carried out under their compliance assurance program for the prior year and the plans for the compliance assurance program during the current year. The report will include information regarding grower interactions (including, but not limited to, on-farm visits, verified tips and complaints, grower meetings and letters), the extent of non-compliance, corrective measures to address the non-compliance, and any follow-up actions taken. Syngenta may elect to coordinate information with other registrants and report collectively the results of compliance assurance programs.

15) Syngenta and the seed corn dealers for Syngenta must allow a review of the compliance records by EPA or by a State pesticide regulatory agency if the State agency can demonstrate that confidential business information, including the names, personal information, and license number of the growers will be protected.

d) Insect Resistance Monitoring and Remedial Action Plans for *Bt*11 x MIR162 Corn

1) The Agency is imposing the following conditions for the Cry1Ab toxin expressed in *Bt*11 corn:

Syngenta will monitor for resistance to Cry1Ab expressed in *Bt*11 x MIR162 corn. The monitoring program shall consist of two approaches: (i) focused population sampling and laboratory testing and (ii) investigation of reports of less-than expected control of labeled insects. Should field-relevant resistance be confirmed, an appropriate resistance management action plan will be implemented.

i) Focused Population Sampling

Syngenta shall annually sample and bioassay populations of the key target pests: *Ostrinia nubilalis* (European corn borer; ECB), *Diatraea grandiosella* (Southwestern corn borer; SWCB), and *Helicoverpa zea* (corn earworm; CEW). Sampling for the target pests will be focused in areas identified as those with the highest risk of resistance development (e.g., where lepidopteran-active *Bt* hybrids are planted on a high proportion of the corn acres, and where the insect species are regarded as key pests of corn). Bioassay methods must be appropriate for the goal of detecting field-relevant shifts in population response to *Bt*11 x MIR162 corn and/or changes in resistance-allele frequency in response to the use of *Bt*11 x MIR162 corn and, as far as possible, should be consistent across sampling years to enable comparisons with historical data.

The number of populations to be collected shall reflect the regional importance of the

insect species as a pest, and specific collection regions will be identified for each pest. For ECB, a minimum of 12 populations across the sampling region will be targeted for collection at each annual sampling. For SWCB, the target will be a minimum of six populations. For CEW, the target will be a minimum of ten populations. Pest populations should be collected from multiple corn-growing states reflective of different geographies and agronomic conditions. To obtain sufficient sensitivity to detect resistance alleles before they become common enough to cause measurable field damage, each population collection shall attempt to target 400 insect genomes (egg masses, larvae, mated females, and/or mixed-sex adults), but a successful population collection will contain a minimum of 100 genomes. It is recognized that it may not be possible to collect the target number of insect populations or genomes due to factors such as natural fluctuations in pest density, environmental conditions, and area-wide pest suppression.

The sampling program and geographic range of collections may be modified as appropriate based on changes in pest importance and for the adoption levels of *Bt*11 x MIR162 corn. The Agency shall be consulted prior to the implementation of such modifications.

Syngenta will report to the Agency by August 31st of each year, beginning in 2010, the results of the population sampling and bioassay monitoring program.

Any incidence of unusually low sensitivity to the Cry1Ab protein in bioassays shall be investigated as soon as possible to understand any field relevance of such a finding. Such investigations shall proceed in a stepwise manner until the field relevance can be either confirmed or refuted, and results of these shall be reported to the Agency annually before August 31st, beginning in 2010. The investigative steps will include:

1. Re-test progeny of the collected population to determine whether the unusual bioassay response is reproducible and heritable. If it is not reproducible and heritable, no further action is required.

2. If the unusual response is reproducible and heritable, progeny of insects that survive the diagnostic concentration will be tested using methods that are representative of exposure to *Bt*11 x MIR162 corn under field conditions. If progeny do not survive to adulthood, any suspected resistance is not field relevant and no further action is required.

3. If insects survive steps 1 and 2, resistance is confirmed, and further steps will be taken to evaluate the resistance. These steps may include:

    - determining the nature of the resistance (i.e., recessive or dominant, and the level of functional dominance);

- estimating the resistance-allele frequency in the original population;
- determining whether the resistance-allele frequency is increasing by analyzing field collections in subsequent years sampled from the same site where the resistance allele(s) was originally collected;
- determining the geographic distribution of the resistance allele by analyzing field collections in subsequent years from sites surrounding the site where the resistance allele(s) was originally collected.

Should field-relevant resistance be confirmed, and the resistance appears to be increasing or spreading, Syngenta will consult with the Agency to develop and implement a case-specific resistance management action plan.

ii) Investigation of Reports of Unexpected Levels of Damage by the Target Pests

Syngenta will follow up on grower, extension specialist or consultant reports of unexpected levels of damage by the lepidopteran pests listed on the pesticide label. Syngenta will instruct its customers to contact them if such incidents occur. Syngenta will investigate all legitimate reports submitted to the company or the company's representatives.

If reports of unexpected levels of damage lead to the suspicion of resistance in any of the key target pests (ECB, SWCB, and CEW), Syngenta will implement the actions described below, based on the following definitions of *suspected resistance* and *confirmed resistance.*

Suspected resistance

EPA defines *suspected resistance* to mean field reports of unexpected levels of insect feeding damage for which:

- the corn in question has been confirmed to be lepidopteran-active *Bt* corn;
- the seed used had the proper percentage of corn expressing *Bt* protein;
- the relevant plant tissues are expressing the expected level of *Bt* protein; and
- it has been ruled out that species not susceptible to the protein could be responsible for the damage, that no climatic or cultural reasons could be responsible for the damage, and that there could be no other reasonable causes for the damage.

The Agency does not interpret *suspected resistance* to mean grower reports of possible control failures or suspicious results from annual insect monitoring assays, nor does the Agency intend that extensive field studies and testing be undertaken to confirm scientifically the presence of insects resistant to *Bt*11 x MIR162 corn in commercial production fields before responsive measures are undertaken.

If resistance is *suspected,* Syngenta will instruct growers to do the following:

- Use alternative control measures in *Bt*11 x MIR162 corn fields in the affected region to control the target pest during the immediate growing season.
- Destroy *Bt*11 x MIR162 corn crop residues in the affected region within one month after harvest with a technique appropriate for local production practices to minimize the possibility of resistant insects over-wintering and contributing to the next season's target pest population.

Additionally, if possible, and prior to the application of alternative control measures or destruction of crop residue, Syngenta will collect samples of the insect population in the affected fields for laboratory rearing and testing. Such rearing and testing shall be conducted as expeditiously as practical.

Confirmed resistance

EPA defines *confirmed resistance* to mean, in the case of field reports of unexpected levels of damage from the key target pests, that all the following criteria are met:

- There is >30% insect survival and commensurate insect feeding in a bioassay, initiated with neonate larvae, that uses methods that are representative of exposure to *Bt* corn hybrids under field conditions (ECB and SWCB only).
- In standardized laboratory bioassays using diagnostic concentrations of the *Bt* protein suited to the target pest in question, the pest exhibits resistance that has a genetic basis and the level of survivorship indicates that there may be a resistance-allele frequency of ≥ 0.1 in the sampled population.
- In standardized laboratory bioassays, the LC_{50} exceeds the upper limit of the 95% confidence interval of the LC_{50} for susceptible populations surveyed both in the original baselines developed for this pest species and in previous years of field monitoring

iii) Response to Confirmed Resistance in a Key Target Pest as the Cause of Unexpected Levels of Damage in the Field

When field resistance is *confirmed* (as defined above), the following steps will be taken by Syngenta:

- EPA will receive notification within 30 days of resistance confirmation;
- Affected customers and extension agents will be notified about confirmed resistance within 30 days;
- Monitoring will be increased in the affected area and local target pest populations will

be sampled annually to determine the extent and impact of resistance;

- If appropriate (depending on the resistant pest species, the extent of resistance, the timing of resistance, and the nature of resistance, and the availability of suitable alternative control measures), alternative control measures will be employed to reduce or control target pest populations in the affected area. Alternative control measures may include advising customers and extension agents in the affected area to incorporate crop residues into the soil following harvest to minimize the possibility of over-wintering insects, and/or applications of chemical insecticides;
- Unless otherwise agreed with EPA, stop sale and distribution of the relevant lepidopteran-active *Bt* corn hybrids in the affected area immediately until an effective local mitigation plan approved by EPA has been implemented;
- Syngenta will develop a case-specific resistance management action plan within 90 days according to the characteristics of the resistance event and local agronomic needs. Syngenta will consult with appropriate stakeholders in the development of the action plan, and the details of such a plan shall be approved by EPA prior to implementation;
- Notify affected parties (e.g., growers, consultants, extension agents, seed distributors, university cooperators and state/federal authorities as appropriate) in the region of the resistance situation and approved action plan; and
- In subsequent growing seasons, maintain sales suspension and alternative resistance management strategies in the affected region(s) for the *Bt* corn hybrids that are affected by the resistant population until an EPA-approved local resistance management plan is in place to mitigate the resistance.

A report on results of resistance monitoring and investigations of damage reports must be submitted to the Agency annually by August 31st each year, beginning in 2010, for the duration of the conditional registration.

2) The Agency is imposing the following conditions for the Vip3Aa20 toxin expressed in MIR162 maize:

A detailed resistance monitoring program and final remedial action plan, integrating standard procedures developed by the ABSTC (as outlined below) and harmonized with resistance monitoring programs and remedial action plans established for previously registered Syngenta *Bt* corn products, for the key target pests of CEW and SWCB must be submitted within 90 days of the date of registration.

Syngenta will monitor for resistance to Vip3Aa20 expressed in *Bt*11 x MIR162 corn. The monitoring program shall consist of two approaches: (i) focused population sampling and laboratory testing and (ii) investigation of reports of less-than expected control of labeled insects. Should field-relevant resistance be confirmed, an appropriate resistance management action plan will be implemented.

i) Focused Population Sampling

Syngenta shall annually sample and bioassay populations of the key target pests: *Diatraea grandiosella* (Southwestern corn borer; SWCB) and *Helicoverpa zea* (corn earworm; CEW). Sampling for the target pests will be focused in areas identified as those with the highest risk of resistance development (e.g., where lepidopteran-active *Bt* hybrids are planted on a high proportion of the corn acres, and where the insect species are regarded as key pests of corn). Bioassay methods must be appropriate for the goal of detecting field-relevant shifts in population response to *Bt*11 x MIR162 corn and/or changes in resistance-allele frequency in response to the use of *Bt*11 x MIR162 corn and, as far as possible, should be consistent across sampling years to enable comparisons with historical data.

The number of populations to be collected shall reflect the regional importance of the insect species as a pest, and specific collection regions will be identified for each pest. For SWCB, the target will be a minimum of six populations. For CEW, the target will be a minimum of ten populations. Pest populations should be collected from multiple corn-growing states reflective of different geographies and agronomic conditions. To obtain sufficient sensitivity to detect resistance alleles before they become common enough to cause measurable field damage, each population collection shall attempt to target 400 insect genomes (egg masses, larvae, mated females, and/or mixed-sex adults), but a successful population collection will contain a minimum of 100 genomes. It is recognized that it may not be possible to collect the target number of insect populations or genomes due to factors such as natural fluctuations in pest density, environmental conditions, and area-wide pest suppression.

The sampling program and geographic range of collections may be modified as appropriate based on changes in pest importance and for the adoption levels of *Bt*11 x MIR162 corn. The Agency shall be consulted prior to the implementation of such modifications.

Syngenta will report to the Agency by August 31st of each year, beginning in 2010, the results of the population sampling and bioassay monitoring program.

Any incidence of unusually low sensitivity to the Vip3Aa20 protein in bioassays shall be investigated as soon as possible to understand any field relevance of such a finding. Such investigations shall proceed in a stepwise manner until the field relevance can be either confirmed or refuted, and results of these shall be reported to the Agency annually before August 31st, beginning in 2010. The investigative steps will include:

1. Re-test progeny of the collected population to determine whether the unusual bioassay response is reproducible and heritable. If it is not reproducible and

heritable, no further action is required.

2. If the unusual response is reproducible and heritable, progeny of insects that survive the diagnostic concentration will be tested using methods that are representative of exposure to *Bt*11 x MIR162 corn under field conditions. If progeny do not survive to adulthood, any suspected resistance is not field relevant and no further action is required.

3. If insects survive steps 1 and 2, resistance is confirmed, and further steps will be taken to evaluate the resistance. These steps may include:

   - determining the nature of the resistance (i.e., recessive or dominant, and the level of functional dominance);
   - estimating the resistance-allele frequency in the original population;
   - determining whether the resistance-allele frequency is increasing by analyzing field collections in subsequent years sampled from the same site where the resistance allele(s) was originally collected;
   - determining the geographic distribution of the resistance allele by analyzing field collections in subsequent years from sites surrounding the site where the resistance allele(s) was originally collected.

Should field-relevant resistance be confirmed, and the resistance appears to be increasing or spreading, Syngenta will consult with the Agency to develop and implement a case-specific resistance management action plan.

ii) Investigation of Reports of Unexpected Levels of Damage by the Target Pests

Syngenta will follow up on grower, extension specialist or consultant reports of unexpected levels of damage by the lepidopteran pests listed on the pesticide label. Syngenta will instruct its customers to contact them if such incidents occur. Syngenta will investigate all legitimate reports submitted to the company or the company's representatives.

If reports of unexpected levels of damage lead to the suspicion of resistance in any of the key target pests (SWCB and CEW), Syngenta will implement the actions described below, based on the following definitions of *suspected resistance* and *confirmed resistance.*

Suspected resistance

EPA defines *suspected resistance* to mean field reports of unexpected levels of insect feeding damage for which:

- the corn in question has been confirmed to be lepidopteran-active *Bt* corn;
- the seed used had the proper percentage of corn expressing *Bt* protein;
- the relevant plant tissues are expressing the expected level of *Bt* protein; and
- it has been ruled out that species not susceptible to the protein could be responsible for the damage, that no climatic or cultural reasons could be responsible for the damage, and that there could be no other reasonable causes for the damage.

The Agency does not interpret *suspected resistance* to mean grower reports of possible control failures or suspicious results from annual insect monitoring assays, nor does the Agency intend that extensive field studies and testing be undertaken to confirm scientifically the presence of insects resistant to *Bt*11 x MIR162 corn in commercial production fields before responsive measures are undertaken.

If resistance is *suspected,* Syngenta will instruct growers to do the following:

- Use alternative control measures in *Bt*11 x MIR162 corn fields in the affected region to control the target pest during the immediate growing season.
- Destroy *Bt*11 x MIR162 corn crop residues in the affected region within one month after harvest with a technique appropriate for local production practices to minimize the possibility of resistant insects over-wintering and contributing to the next season's target pest population.

Additionally, if possible, and prior to the application of alternative control measures or destruction of crop residue, Syngenta will collect samples of the insect population in the affected fields for laboratory rearing and testing. Such rearing and testing shall be conducted as expeditiously as practical.

Confirmed resistance

EPA defines *confirmed resistance* to mean, in the case of field reports of unexpected levels of damage from the key target pests, that all the following criteria are met:

- There is >30% insect survival and commensurate insect feeding in a bioassay, initiated with neonate larvae, that uses methods that are representative of exposure to *Bt* corn hybrids under field conditions (SWCB only).
- In standardized laboratory bioassays using diagnostic concentrations of the *Bt* protein suited to the target pest in question, the pest exhibits resistance that has a genetic basis and the level of survivorship indicates that there may be a resistance-allele frequency of ≥ 0.1 in the sampled population.
- In standardized laboratory bioassays, the LC_{50} exceeds the upper limit of the 95% confidence interval of the LC_{50} for susceptible populations surveyed both in the original baselines developed for this pest species and in previous years of field

monitoring.

iii) Response to Confirmed Resistance in a Key Target Pest as the Cause of Unexpected Levels of Damage in the Field

When field resistance is *confirmed* (as defined above), the following steps will be taken by Syngenta:

- EPA will receive notification within 30 days of resistance confirmation;
- Affected customers and extension agents will be notified about confirmed resistance within 30 days;
- Monitoring will be increased in the affected area and local target pest populations will be sampled annually to determine the extent and impact of resistance;
- If appropriate (depending on the resistant pest species, the extent of resistance, the timing of resistance, and the nature of resistance, and the availability of suitable alternative control measures), alternative control measures will be employed to reduce or control target pest populations in the affected area. Alternative control measures may include advising customers and extension agents in the affected area to incorporate crop residues into the soil following harvest to minimize the possibility of over-wintering insects, and/or applications of chemical insecticides;
- Unless otherwise agreed with EPA, stop sale and distribution of the relevant lepidopteran-active *Bt* corn hybrids in the affected area immediately until an effective local mitigation plan approved by EPA has been implemented;
- Syngenta will develop a case-specific resistance management action plan within 90 days according to the characteristics of the resistance event and local agronomic needs. Syngenta will consult with appropriate stakeholders in the development of the action plan, and the details of such a plan shall be approved by EPA prior to implementation;
- Notify affected parties (e.g., growers, consultants, extension agents, seed distributors, university cooperators and state/federal authorities as appropriate) in the region of the resistance situation and approved action plan; and
- In subsequent growing seasons, maintain sales suspension and alternative resistance management strategies in the affected region(s) for the *Bt* corn hybrids that are affected by the resistant population until an EPA-approved local resistance management plan is in place to mitigate the resistance.

A report on results of resistance monitoring and investigations of damage reports must be submitted to the Agency annually by August 31st each year, beginning in 2010, for the duration of the conditional registration.

e) Annual Reporting Requirements for *Bt*11 x MIR162 Corn

1) Annual Sales: reported and summed by state (county level data available by request) January 31st each year, beginning in 2010;

2) Grower Agreements: number of units of Bt11 x MIR162 corn seed shipped or sold and not returned, and the number of such units that were sold to persons who have signed grower agreements, January 31st each year, beginning in 2010;

3) Grower Education: substantive changes to education program completed previous year, January 31st each year, beginning in 2010;

4) Compliance Assurance Program: compliance assurance program activities and results for the prior year and plans for the compliance assurance program for the current year, January 31st each year, beginning in 2010;

5) Compliance Survey Results: results of annual surveys for the prior year and survey plans for the current year; full report January 31st each year, beginning in 2010;

6) Insect Resistance Monitoring Results: results of monitoring and investigations of damage reports, August 31st each year, beginning in 2010.

3. *Bt*11 x MIR162 x MIR604 Corn

The following terms and/or conditions are required for the FIFRA section 3(c)(7)(C) registration of *Bt*11 x MIR162 x MIR604 corn:

1) The subject registration will automatically expire at midnight on December 31, 2011.

2) The subject registration will be limited to Cry1Ab (*Bacillus thuringiensis* Cry1Ab delta-endotoxin protein and the genetic material necessary for its production [*via* elements of vector pZO1502] in Event *Bt*11 corn [OECD Unique Identifier: SYN-BTØ11-1]) x Vip3Aa20 (*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]) x mCry3A (modified Cry3A protein and the genetic material necessary for its production [*via* elements of vector pZM26] in Event MIR604 corn [OECD Unique Identifier: SYN-IR6Ø4-5]) for use in field corn.

3) 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.

4) Syngenta will submit/cite all data required to support the individual plant-incorporated protectants in YieldGard® Insect Resistant Corn, MIR162 maize, and Agrisure® RW Rootworm-Protected Corn within the time frames required by the terms and conditions of EPA Registration Numbers 67979-1, 67979-14, and 67979-5, respectively:

Study Type ****Registration**	**Required Data**	**Due Date**
Residue Analytical Method – Plants (OPPTS 860.1340) **MIR162 maize	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 provide to the 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 (OPPTS 885.4240) **MIR162 maize	A 7–14 day *Daphnia* study as per the OPPTS 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 *Daphnia* study.	November 1, 2009
Insect Resistance Management – Annual Reporting **MIR162 maize	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
Simulated or Actual Field Tests – Non-Target Invertebrates **Agrisure® RW Rootworm-Protected Corn	Three (3) year full-scale field or semi-field studies for evaluation of mCry3A Event MIR604 corn exposure on non-target invertebrates must be conducted and a final report submitted. Full-scale field experiments must be appropriately designed to provide a measure of ecological impacts (larger fields, more replicates, more samples per plot based on recommendations of the August 2002 Scientific Advisory Panel [SAP] and the subsequent relevant research on appropriate study design).	January 31, 2011
Field Degradation Studies **Agrisure® RW Rootworm-Protected Corn	Field degradation studies evaluating accumulation and persistence of mCry3A in several soils and various strata must be conducted and a final report, regarding data from fields that have had three continuous years of cultivation of Event MIR604 corn, submitted. Representative fields must have been planted with mCry3A corn, include both conventional tillage and no-till samples, and be harvested under typical agronomic conditions. Sampling must continue until the limit of detection is reached. Studies should include soils with high levels of a variety of clays. Both enzyme-linked immunosorbent assay (ELISA) and insect bioassays need to be conducted to determine if mCry3A is accumulating or persisting in soil samples.	January 31, 2011

5) Syngenta must submit the following data and/or information in the time frames listed:

Study Type	Required Data	Due Date
Insect Resistance Management – Dose	Because of the potential for synergistic interactions between plant-incorporated protectants in a stacked product, field efficacy studies and/or a protein expression report for SWCB, which show that *Bt*11 x MIR162 x MIR604 corn has the same dose profile as its single trait products, must be submitted as confirmatory data.	March 1, 2010
Insect Resistance Management – Grower Agreement	A copy of the grower agreement, associated stewardship documents, and written description of a system, which assures that growers will sign grower agreements and persons purchasing *Bt*11 x MIR162 x MIR604 corn will annually affirm that they are contractually bound to comply with the requirements of the IRM program, must be submitted.	Within 90 days of the date of registration
Insect Resistance Management – Compliance Monitoring Program	A CAP for *Bt*11 x MIR162 x MIR604 corn must be submitted and must include a "phased compliance approach" that outlines instances of non-compliance to the IRM requirements and options of responding to non-compliant growers. This compliance assurance program should be harmonized with compliance assurance programs already in place for previously registered Syngenta *Bt* corn products.	Within 90 days of the date of registration
Insect Resistance Management – Resistance Monitoring	Baseline susceptibility and diagnostic concentration determinations for SWCB and CEW to Vip3Aa20 must be submitted.	August 31, 2010
Insect Resistance Management – Resistance Monitoring	A detailed Vip3Aa20 resistance monitoring plan, integrating standard procedures developed by the ABSTC and similar in structure to those established for previously registered Syngenta *Bt* corn products, for the key target pests of CEW and SWCB must be submitted.	Within 90 days of the date of registration
Insect Resistance Management – Resistance Monitoring	A revised mCry3A resistance monitoring program that incorporates *Bt*11 x MIR162 x MIR604 corn must be submitted. <u>Consideration for corn rootworm (CRW):</u> In addition to mortality assays, consider utilizing sublethal bioassays (e.g., head capsule measurements) and molecular marker methods for CRW monitoring.	Within 90 days of the date of registration
Insect Resistance Management – Resistance Monitoring	Submit data generated by the following actions: (a) initiate establishment of CRW strains that are resistant to mCry3A and investigate the nature, inheritance, and fitness costs of specific mechanisms of resistance to mCry3A, (b) study the behavioral deterrence (avoidance) mechanism further, and (c) continue studies on the biological impact of CRW adults surviving on corn expressing the mCry3A toxin.	January 31, 2010

Study Type	Required Data	Due Date
Insect Resistance Management – Resistance Monitoring	Develop, validate, and submit an appropriate discriminating or diagnostic dose assay for the mCry3A resistance monitoring program.	January 31, 2010
Insect Resistance Management – Resistance Monitoring	Finalize and submit rootworm damage guidelines for the mCry3A resistance monitoring program.	January 31, 2010
Insect Resistance Management – Remedial Action Plan	A final remedial action plan for the Vip3Aa20 toxin expressed in *Bt*11 x MIR162 x MIR604 corn, integrating the standard procedures developed by the ABSTC and harmonized with remedial action plans established for previously registered Syngenta *Bt* corn products, must be submitted.	Within 90 days of the date of registration

6) The insect resistance management terms and conditions for *Bt*11 x MIR162 x MIR604 corn are as follows.

The required IRM program for *Bt*11 x MIR162 x MIR604 corn must have the following elements:

- Requirements relating to creation of a non-*Bt* corn and/or non-lepidopteran-resistant *Bt* corn refuge in conjunction with the planting of any acreage of *Bt*11 x MIR162 x MIR604 corn;
- Requirements for Syngenta to prepare and require *Bt*11 x MIR162 x MIR604 corn users to sign "grower agreements," which impose binding contractual obligations on the grower to comply with the refuge requirements;
- Requirements regarding programs to educate growers about IRM requirements;
- Requirements regarding programs to evaluate and promote growers' compliance with IRM requirements;
- Requirements regarding programs to evaluate whether there are statistically significant and biologically relevant changes in target insect susceptibility to Vip3Aa20, Cry1Ab, and/or mCry3A proteins in the target insects;
- Requirements regarding a "remedial action plan," which contains measures Syngenta would take in the event that any field-relevant insect resistance was detected as well as to report on activity under the plan to EPA;
- Annual reports on units sold by state (units sold by county level will be made available to the Agency upon request), IRM grower agreements results, and the compliance assurance program including the educational program on or before January 31st each year, beginning in 2010.

a) Refuge Requirements for *Bt*11 x MIR162 x MIR604 Field Corn

These refuge requirements do not apply to seed increase/propagation of inbred and hybrid seed corn up to a total of 20,000 acres per county and up to a combined U.S. total of 250,000 acres per PIP active ingredient per registrant per year.

Grower agreements (also known as stewardship agreements) will specify that growers must adhere to the refuge requirements as described in the grower guide/product use guide and/or in supplements to the grower guide/product use guide.

Two options for deployment of the refuge are available to growers.

The first option is planting a common refuge for both corn borers and corn rootworms. The common refuge must be planted with corn hybrids that do not contain *Bt* technologies for the control of corn rootworms or corn borers. The refuge area must represent at least 20% of the grower's corn acres (i.e., sum of *Bt*11 x MIR162 x MIR604 corn acres and refuge acres). It must be planted as a block adjacent to the *Bt*11 x MIR162 x MIR604 corn field, perimeter strips, or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least four consecutive rows wide. If the common refuge is planted on rotated ground, then *Bt*11 x MIR162 x MIR604 corn must also be planted on rotated ground. If the common refuge is planted in continuous corn, the *Bt*11 x MIR162 x MIR604 corn field may be planted on either continuous or rotated land. The common refuge can be treated with a soil-applied or seed-applied insecticide to control rootworm larvae and other soil pests. The refuge can also be treated with a non-*Bt* foliar insecticide for control of late season pests, if pest pressure reaches an economic threshold for damage; however, if rootworm adults are present at the time of foliar applications, then the *Bt*11 x MIR162 x MIR604 corn field must be treated in a similar manner. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents or crop consultants). Pests other than adult corn rootworms can be treated with an appropriate pest-labeled insecticide on the common refuge acres without treating the *Bt*11 x MIR162 x MIR604 corn acres only if treatment occurs when adult corn rootworms are not present. Pests on the *Bt*11 x MIR162 x MIR604 corn acres can be treated as needed without having to treat the common refuge.

The second option is planting separate refuge areas for corn borers and corn rootworms. The corn borer refuge must be planted with a non-*Bt*/lepidopteran-protected hybrid, must represent at least 20% of the grower's corn acres (i.e., sum of *Bt*11 x MIR162 x MIR604 corn acres and corn borer refuge acres), and must be planted within ½ mile of the *Bt*11 x MIR162 x MIR604 corn field. Refuge planting options include separate fields, blocks within fields (e.g., along the edges or headlands), perimeter strips, or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least four consecutive rows wide. The corn borer refuge can be treated with a soil-applied or seed-applied insecticide for corn rootworm larval control or a non-*Bt* foliar-applied insecticide for corn borer control, if pest pressure

reaches an economic threshold for damage. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents or crop consultants).

The corn rootworm refuge must be planted with a non-*Bt*/corn rootworm-protected hybrid, but can be planted with *Bt* corn hybrids that control corn borers. The corn rootworm refuge must represent at least 20% of the grower's corn acres (i.e., sum of *Bt*11 x MIR162 x MIR604 corn acres and corn rootworm refuge acres) and must be planted as an adjacent block, perimeter strips, or in-field strips. If perimeter or in-field strips are implemented, the strips must be at least four consecutive rows wide. If the rootworm refuge is planted on rotated ground, then *Bt*11 x MIR162 x MIR604 corn must also be planted on rotated ground. If the rootworm refuge is planted in continuous corn, the *Bt*11 x MIR162 x MIR604 corn field may be planted on either continuous or rotated land. More generally, the rootworm refuge should utilize comparable agronomic practices as the *Bt*11 x MIR162 x MIR604 corn acres. The corn rootworm refuge can be treated with a soil-applied or seed-applied insecticide to control rootworm larvae and other soil pests. The refuge can also be treated with a non-*Bt* foliar insecticide for control of late season pests; however, if rootworm adults are present at the time of foliar applications, then the *Bt*11 x MIR162 x MIR604 corn field must be treated in a similar manner. Pests other than adult corn rootworms can be treated on the rootworm refuge acres without treating the *Bt*11 x MIR162 x MIR604 corn acres only if treatment occurs when adult corn rootworms are not present or if a pesticide without activity against adult corn rootworms is used. Pests on the *Bt*11 x MIR162 x MIR604 corn acres can be treated as needed without having to treat the rootworm refuge.

b) Grower Agreement for *Bt*11 x MIR162 x MIR604 Corn

1) Persons purchasing *Bt*11 x MIR162 x MIR604 corn must sign a grower agreement. The term "grower agreement" refers to any grower purchase contract, license agreement, or similar legal document.

2) The grower agreement and/or specific stewardship documents referenced in the grower agreement must clearly set forth the terms of the current IRM program. By signing the grower agreement, a grower must be contractually bound to comply with the requirements of the IRM program.

3) Syngenta must implement a system (equivalent to what is already approved for previously registered Syngenta *Bt* corn products), which is reasonably likely to assure that persons purchasing *Bt*11 x MIR162 x MIR604 corn will affirm annually that they are contractually bound to comply with the requirements of the IRM program. A description of the system must be submitted to EPA within 90 days from the date of registration.

4) Syngenta must use an approved grower agreement and must submit to EPA, within 90 days from the date of registration, a copy of that agreement and any specific stewardship documents referenced in the grower agreement. If Syngenta wishes to change any part of the grower agreement or any specific stewardship documents referenced in the grower agreement that would affect either the content of the IRM program or the legal enforceability of the provisions of the agreement relating to the IRM program, thirty days prior to implementing a proposed change, Syngenta must submit to EPA the text of such changes to ensure that it is consistent with the terms and conditions of this registration.

5) Syngenta must implement an approved system (equivalent to what is already approved for previously registered Syngenta *Bt* corn products), which is reasonably likely to assure that persons purchasing *Bt*11 x MIR162 x MIR604 corn sign grower agreement(s). A description of the system must be submitted to EPA within 90 days from the date of registration.

6) Syngenta shall maintain records of all *Bt*11 x MIR162 x MIR604 corn grower agreements for a period of three years from December 31st of the year in which the agreement was signed.

7) Beginning on January 31, 2010 and annually thereafter, Syngenta shall provide EPA with a report on the number of units of *Bt*11 x MIR162 x MIR604 corn seed shipped and not returned, and the number of such units that were sold to persons who have signed grower agreements. The report shall cover the time frame of a twelve-month period. Note: The first report shall contain the specified information from the time frame starting with the date of registration and extending through the 2009 growing season.

8) Syngenta must allow a review of the grower agreements and grower agreement records by EPA or by a State pesticide regulatory agency if the State agency can demonstrate that confidential business information, including names, personal information, and grower license number, will be protected.

c) IRM Education and IRM Compliance Monitoring Program for *Bt*11 x MIR162 x MIR604 Corn

1) Syngenta must design and implement a comprehensive, ongoing IRM education program designed to convey to *Bt*11 x MIR162 x MIR604 corn users the importance of complying with the IRM program. The education program shall involve the use of multiple media, e.g. face-to-face meetings, mailing written materials, EPA-reviewed language on IRM requirements on the bag or bag tag, and electronic communications such as by internet, radio, or television commercials. Copies of the materials will be provided to EPA for their records. The program shall involve at least one written communication annually to

each *Bt*11 x MIR162 x MIR604 corn user separate from the grower technical guide. The communication shall inform the user of the current IRM requirements. Syngenta shall coordinate its education program with the educational efforts of other registrants and other organizations, such as the National Corn Growers Association and state extension programs.

2) Annually, Syngenta shall revise, and expand as necessary, its education program to take into account the information collected through the compliance survey required under paragraph 6 and from other sources. The changes shall address aspects of grower compliance that are not sufficiently high.

3) Beginning January 31, 2010, Syngenta must provide a report to EPA summarizing the activities it carried out under its education program for the prior year. Annually thereafter, Syngenta must provide EPA any substantive changes to its grower education activities as part of the overall IRM compliance assurance program report. Syngenta must either submit a separate report or contribute to the report from the industry working group, ABSTC.

4) Syngenta must design and implement an ongoing IRM compliance assurance program designed to evaluate the extent to which growers purchasing *Bt*11 x MIR162 x MIR604 corn are complying with the IRM program and that takes such actions as are reasonably needed to assure that growers who have not complied with the program either do so in the future or lose their access to *Bt*11 x MIR162 x MIR604 corn. Syngenta shall coordinate with other *Bt* corn registrants in designing and implementing its compliance assurance program and integrate this registration into the current compliance assurance program used for their other *Bt* corn PIPs. Syngenta must prepare and submit within 90 days of the date of registration a written description of the compliance assurance program. Other required features of the program are described in paragraphs 5–15 below.

5) Syngenta must establish and publicize a "phased compliance approach," i.e., a guidance document that indicates how they will address instances of non-compliance with the terms of the IRM program and general criteria for choosing among options for responding to any non-compliant growers. While recognizing that for reasons of difference in business practices there are needs for flexibility between different companies, Syngenta must use a consistent set of standards for responding to non-compliance. The options shall include withdrawal of the right to purchase *Bt*11 x MIR162 x MIR604 corn for an individual grower or for all growers in a specific region. An individual grower found to be significantly out of compliance two years in a row would be denied sales of *Bt*11 x MIR162 x MIR604 corn the next year. Similarly, seed dealers who are not fulfilling their obligations to inform/educate growers of their IRM obligations will lose their opportunity to sell *Bt*11 x MIR162 x MIR604 corn.

6) The IRM compliance assurance program shall include an annual survey, conducted by an independent third party, of a statistically representative sample of growers of *Bt*11 x MIR162 x MIR604 corn who plant the vast majority of all corn in the United States and in areas in which the selection intensity is the greatest. The survey shall consider only those growers who plant 200 or more acres of corn in the Corn Belt or who plant 100 or more acres of corn in corn-cotton growing areas. The survey shall measure the degree of compliance with the IRM program by growers in different regions of the country and consider the potential impact of non-response. The sample size and geographical resolution may be adjusted annually, based upon input from independent marketing research firms and academic scientists, to allow analysis of compliance behavior within regions or between regions. The sample size must provide a reasonable sensitivity for comparing results across the United States.

7) The survey shall be designed to provide an understanding of any difficulties growers encounter in implementing IRM requirements. An analysis of the survey results must include the reasons, extent, and potential biological significance of any implementation deviations.

8) The survey shall be designed to obtain grower feedback on the usefulness of specific educational tools and initiatives.

9) Syngenta shall provide a final written summary of the results of the prior year's survey (together with a description of the regions, the methodology used, and the supporting data) to EPA by January 31st of each year, beginning with 2010. Syngenta shall confer with other registrants and EPA on the design and content of the survey prior to its implementation.

10) Annually, Syngenta shall revise, and expand as necessary, its compliance assurance program to take into account the information collected through the compliance survey (required under paragraphs 6 through 8) and from other sources. The changes shall address aspects of grower compliance that are not sufficiently high. Syngenta must confer with the Agency prior to adopting any changes.

11) Syngenta shall conduct an annual on-farm assessment program. Syngenta shall train its representatives who make on-farm visits with growers of *Bt*11 x MIR162 x MIR604 corn to perform assessments of compliance with IRM requirements. There is no minimum corn acreage size for this program. Therefore, growers will be selected for this program from across all farm sizes. In the event that any of these visits result in the identification of a grower who is not in compliance with the IRM program, Syngenta shall take appropriate action, consistent with its "phased compliance approach" to promote compliance.

12) Syngenta shall carry out a program for investigating legitimate "tips and complaints" that its growers are not in compliance with the IRM program. Whenever an investigation results in the identification of a grower who is not in compliance with the IRM program, Syngenta shall take appropriate action, consistent with its "phased compliance approach."

13) If a grower, who purchases *Bt*11 x MIR162 x MIR604 corn for planting, was specifically identified as not being in compliance during the previous year, Syngenta shall visit with the grower and evaluate whether that the grower is in compliance with the IRM program for the current year.

14) Beginning January 31, 2010 and annually thereafter, Syngenta shall provide a report to EPA summarizing the activities carried out under their compliance assurance program for the prior year and the plans for the compliance assurance program during the current year. The report will include information regarding grower interactions (including, but not limited to, on-farm visits, verified tips and complaints, grower meetings and letters), the extent of non-compliance, corrective measures to address the non-compliance, and any follow-up actions taken. Syngenta may elect to coordinate information with other registrants and report collectively the results of compliance assurance programs.

15) Syngenta and the seed corn dealers for Syngenta must allow a review of the compliance records by EPA or by a State pesticide regulatory agency if the State agency can demonstrate that confidential business information, including the names, personal information, and license number of the growers will be protected.

d) Insect Resistance Monitoring and Remedial Action Plans for *Bt*11 x MIR162 x MIR604 Corn

1) The Agency is imposing the following conditions for the Cry1Ab toxin expressed in *Bt*11 corn:

Syngenta will monitor for resistance to Cry1Ab expressed in *Bt*11 x MIR162 x MIR604 corn. The monitoring program shall consist of two approaches: (i) focused population sampling and laboratory testing and (ii) investigation of reports of less-than expected control of labeled insects. Should field-relevant resistance be confirmed, an appropriate resistance management action plan will be implemented.

i) Focused Population Sampling

Syngenta shall annually sample and bioassay populations of the key target pests: *Ostrinia nubilalis* (European corn borer; ECB), *Diatraea grandiosella* (Southwestern corn borer; SWCB), and *Helicoverpa zea* (corn earworm; CEW). Sampling for the target pests will

be focused in areas identified as those with the highest risk of resistance development (e.g., where lepidopteran-active *Bt* hybrids are planted on a high proportion of the corn acres, and where the insect species are regarded as key pests of corn). Bioassay methods must be appropriate for the goal of detecting field-relevant shifts in population response to *Bt*11 x MIR162 x MIR604 corn and/or changes in resistance-allele frequency in response to the use of *Bt*11 x MIR162 x MIR604 corn and, as far as possible, should be consistent across sampling years to enable comparisons with historical data.

The number of populations to be collected shall reflect the regional importance of the insect species as a pest, and specific collection regions will be identified for each pest. For ECB, a minimum of 12 populations across the sampling region will be targeted for collection at each annual sampling. For SWCB, the target will be a minimum of six populations. For CEW, the target will be a minimum of ten populations. Pest populations should be collected from multiple corn-growing states reflective of different geographies and agronomic conditions. To obtain sufficient sensitivity to detect resistance alleles before they become common enough to cause measurable field damage, each population collection shall attempt to target 400 insect genomes (egg masses, larvae, mated females, and/or mixed-sex adults), but a successful population collection will contain a minimum of 100 genomes. It is recognized that it may not be possible to collect the target number of insect populations or genomes due to factors such as natural fluctuations in pest density, environmental conditions, and area-wide pest suppression.

The sampling program and geographic range of collections may be modified as appropriate based on changes in pest importance and for the adoption levels of *Bt*11 x MIR162 x MIR604 corn. The Agency shall be consulted prior to the implementation of such modifications.

Syngenta will report to the Agency by August 31st of each year, beginning in 2010, the results of the population sampling and bioassay monitoring program.

Any incidence of unusually low sensitivity to the Cry1Ab protein in bioassays shall be investigated as soon as possible to understand any field relevance of such a finding. Such investigations shall proceed in a stepwise manner until the field relevance can be either confirmed or refuted, and results of these shall be reported to the Agency annually before August 31st, beginning in 2010. The investigative steps will include:

1. Re-test progeny of the collected population to determine whether the unusual bioassay response is reproducible and heritable. If it is not reproducible and heritable, no further action is required.

2. If the unusual response is reproducible and heritable, progeny of insects that survive the diagnostic concentration will be tested using methods that are

representative of exposure to *Bt*11 x MIR162 x MIR604 corn under field conditions. If progeny do not survive to adulthood, any suspected resistance is not field relevant and no further action is required.

3. If insects survive steps 1 and 2, resistance is confirmed, and further steps will be taken to evaluate the resistance. These steps may include:

   - determining the nature of the resistance (i.e., recessive or dominant, and the level of functional dominance);
   - estimating the resistance-allele frequency in the original population;
   - determining whether the resistance-allele frequency is increasing by analyzing field collections in subsequent years sampled from the same site where the resistance allele(s) was originally collected;
   - determining the geographic distribution of the resistance allele by analyzing field collections in subsequent years from sites surrounding the site where the resistance allele(s) was originally collected.

Should field-relevant resistance be confirmed, and the resistance appears to be increasing or spreading, Syngenta will consult with the Agency to develop and implement a case-specific resistance management action plan.

ii) Investigation of Reports of Unexpected Levels of Damage by the Target Pests

Syngenta will follow up on grower, extension specialist or consultant reports of unexpected levels of damage by the lepidopteran pests listed on the pesticide label. Syngenta will instruct its customers to contact them if such incidents occur. Syngenta will investigate all legitimate reports submitted to the company or the company's representatives.

If reports of unexpected levels of damage lead to the suspicion of resistance in any of the key target pests (ECB, SWCB, and CEW), Syngenta will implement the actions described below, based on the following definitions of *suspected resistance* and *confirmed resistance.*

Suspected resistance

EPA defines *suspected resistance* to mean field reports of unexpected levels of insect feeding damage for which:

- the corn in question has been confirmed to be lepidopteran-active *Bt* corn;
- the seed used had the proper percentage of corn expressing *Bt* protein;
- the relevant plant tissues are expressing the expected level of *Bt* protein; and

- it has been ruled out that species not susceptible to the protein could be responsible for the damage, that no climatic or cultural reasons could be responsible for the damage, and that there could be no other reasonable causes for the damage.

The Agency does not interpret *suspected resistance* to mean grower reports of possible control failures or suspicious results from annual insect monitoring assays, nor does the Agency intend that extensive field studies and testing be undertaken to confirm scientifically the presence of insects resistant to *Bt*11 x MIR162 x MIR604 corn in commercial production fields before responsive measures are undertaken.

If resistance is *suspected,* Syngenta will instruct growers to do the following:

- Use alternative control measures in *Bt*11 x MIR162 x MIR604 corn fields in the affected region to control the target pest during the immediate growing season.
- Destroy *Bt*11 x MIR162 x MIR604 corn crop residues in the affected region within one month after harvest with a technique appropriate for local production practices to minimize the possibility of resistant insects over-wintering and contributing to the next season's target pest population.

Additionally, if possible, and prior to the application of alternative control measures or destruction of crop residue, Syngenta will collect samples of the insect population in the affected fields for laboratory rearing and testing. Such rearing and testing shall be conducted as expeditiously as practical.

Confirmed resistance

EPA defines *confirmed resistance* to mean, in the case of field reports of unexpected levels of damage from the key target pests, that all the following criteria are met:

- There is >30% insect survival and commensurate insect feeding in a bioassay, initiated with neonate larvae, that uses methods that are representative of exposure to *Bt* corn hybrids under field conditions (ECB and SWCB only).
- In standardized laboratory bioassays using diagnostic concentrations of the *Bt* protein suited to the target pest in question, the pest exhibits resistance that has a genetic basis and the level of survivorship indicates that there may be a resistance-allele frequency of ≥ 0.1 in the sampled population.
- In standardized laboratory bioassays, the LC_{50} exceeds the upper limit of the 95% confidence interval of the LC_{50} for susceptible populations surveyed both in the original baselines developed for this pest species and in previous years of field monitoring

iii) Response to Confirmed Resistance in a Key Target Pest as the Cause of Unexpected

Levels of Damage in the Field

When field resistance is *confirmed* (as defined above), the following steps will be taken by Syngenta:

- EPA will receive notification within 30 days of resistance confirmation;
- Affected customers and extension agents will be notified about confirmed resistance within 30 days;
- Monitoring will be increased in the affected area and local target pest populations will be sampled annually to determine the extent and impact of resistance;
- If appropriate (depending on the resistant pest species, the extent of resistance, the timing of resistance, and the nature of resistance, and the availability of suitable alternative control measures), alternative control measures will be employed to reduce or control target pest populations in the affected area. Alternative control measures may include advising customers and extension agents in the affected area to incorporate crop residues into the soil following harvest to minimize the possibility of over-wintering insects, and/or applications of chemical insecticides;
- Unless otherwise agreed with EPA, stop sale and distribution of the relevant lepidopteran-active *Bt* corn hybrids in the affected area immediately until an effective local mitigation plan approved by EPA has been implemented;
- Syngenta will develop a case-specific resistance management action plan within 90 days according to the characteristics of the resistance event and local agronomic needs. Syngenta will consult with appropriate stakeholders in the development of the action plan, and the details of such a plan shall be approved by EPA prior to implementation;
- Notify affected parties (e.g., growers, consultants, extension agents, seed distributors, university cooperators and state/federal authorities as appropriate) in the region of the resistance situation and approved action plan; and
- In subsequent growing seasons, maintain sales suspension and alternative resistance management strategies in the affected region(s) for the *Bt* corn hybrids that are affected by the resistant population until an EPA-approved local resistance management plan is in place to mitigate the resistance.

A report on results of resistance monitoring and investigations of damage reports must be submitted to the Agency annually by August 31st each year, beginning in 2010, for the duration of the conditional registration.

2) The Agency is imposing the following conditions for the Vip3Aa20 toxin expressed in MIR162 maize:

A detailed resistance monitoring program and final remedial action plan, integrating standard procedures developed by the ABSTC (as outlined below) and harmonized with

resistance monitoring programs and remedial action plans established for previously registered Syngenta *Bt* corn products, for the key target pests of CEW and SWCB must be submitted within 90 days of the date of registration.

Syngenta will monitor for resistance to Vip3Aa20 expressed in *Bt*11 x MIR162 x MIR604 corn. The monitoring program shall consist of two approaches: (i) focused population sampling and laboratory testing and (ii) investigation of reports of less-than expected control of labeled insects. Should field-relevant resistance be confirmed, an appropriate resistance management action plan will be implemented.

i) Focused Population Sampling

Syngenta shall annually sample and bioassay populations of the key target pests: *Diatraea grandiosella* (Southwestern corn borer; SWCB) and *Helicoverpa zea* (corn earworm; CEW). Sampling for the target pests will be focused in areas identified as those with the highest risk of resistance development (e.g., where lepidopteran-active *Bt* hybrids are planted on a high proportion of the corn acres, and where the insect species are regarded as key pests of corn). Bioassay methods must be appropriate for the goal of detecting field-relevant shifts in population response to *Bt*11 x MIR162 x MIR604 corn and/or changes in resistance-allele frequency in response to the use of *Bt*11 x MIR162 x MIR604 corn and, as far as possible, should be consistent across sampling years to enable comparisons with historical data.

The number of populations to be collected shall reflect the regional importance of the insect species as a pest, and specific collection regions will be identified for each pest. For SWCB, the target will be a minimum of six populations. For CEW, the target will be a minimum of ten populations. Pest populations should be collected from multiple corn-growing states reflective of different geographies and agronomic conditions. To obtain sufficient sensitivity to detect resistance alleles before they become common enough to cause measurable field damage, each population collection shall attempt to target 400 insect genomes (egg masses, larvae, mated females, and/or mixed-sex adults), but a successful population collection will contain a minimum of 100 genomes. It is recognized that it may not be possible to collect the target number of insect populations or genomes due to factors such as natural fluctuations in pest density, environmental conditions, and area-wide pest suppression.

The sampling program and geographic range of collections may be modified as appropriate based on changes in pest importance and for the adoption levels of *Bt*11 x MIR162 x MIR604 corn. The Agency shall be consulted prior to the implementation of such modifications.

Syngenta will report to the Agency by August 31st of each year, beginning in 2010, the

results of the population sampling and bioassay monitoring program.

Any incidence of unusually low sensitivity to the Vip3Aa20 protein in bioassays shall be investigated as soon as possible to understand any field relevance of such a finding. Such investigations shall proceed in a stepwise manner until the field relevance can be either confirmed or refuted, and results of these shall be reported to the Agency annually before August 31st, beginning in 2010. The investigative steps will include:

1. Re-test progeny of the collected population to determine whether the unusual bioassay response is reproducible and heritable. If it is not reproducible and heritable, no further action is required.

2. If the unusual response is reproducible and heritable, progeny of insects that survive the diagnostic concentration will be tested using methods that are representative of exposure to *Bt*11 x MIR162 x MIR604 corn under field conditions. If progeny do not survive to adulthood, any suspected resistance is not field relevant and no further action is required.

3. If insects survive steps 1 and 2, resistance is confirmed, and further steps will be taken to evaluate the resistance. These steps may include:

    - determining the nature of the resistance (i.e., recessive or dominant, and the level of functional dominance);
    - estimating the resistance-allele frequency in the original population;
    - determining whether the resistance-allele frequency is increasing by analyzing field collections in subsequent years sampled from the same site where the resistance allele(s) was originally collected;
    - determining the geographic distribution of the resistance allele by analyzing field collections in subsequent years from sites surrounding the site where the resistance allele(s) was originally collected.

Should field-relevant resistance be confirmed, and the resistance appears to be increasing or spreading, Syngenta will consult with the Agency to develop and implement a case-specific resistance management action plan.

ii) Investigation of Reports of Unexpected Levels of Damage by the Target Pests

Syngenta will follow up on grower, extension specialist or consultant reports of unexpected levels of damage by the lepidopteran pests listed on the pesticide label. Syngenta will instruct its customers to contact them if such incidents occur. Syngenta will investigate all legitimate reports submitted to the company or the company's representatives.

If reports of unexpected levels of damage lead to the suspicion of resistance in any of the key target pests (SWCB and CEW), Syngenta will implement the actions described below, based on the following definitions of *suspected resistance* and *confirmed resistance.*

Suspected resistance

EPA defines *suspected resistance* to mean field reports of unexpected levels of insect feeding damage for which:

- the corn in question has been confirmed to be lepidopteran-active *Bt* corn;
- the seed used had the proper percentage of corn expressing *Bt* protein;
- the relevant plant tissues are expressing the expected level of *Bt* protein; and
- it has been ruled out that species not susceptible to the protein could be responsible for the damage, that no climatic or cultural reasons could be responsible for the damage, and that there could be no other reasonable causes for the damage.

The Agency does not interpret *suspected resistance* to mean grower reports of possible control failures or suspicious results from annual insect monitoring assays, nor does the Agency intend that extensive field studies and testing be undertaken to confirm scientifically the presence of insects resistant to *Bt*11 x MIR162 x MIR604 corn in commercial production fields before responsive measures are undertaken.

If resistance is *suspected,* Syngenta will instruct growers to do the following:

- Use alternative control measures in *Bt*11 x MIR162 x MIR604 corn fields in the affected region to control the target pest during the immediate growing season.
- Destroy *Bt*11 x MIR162 x MIR604 corn crop residues in the affected region within one month after harvest with a technique appropriate for local production practices to minimize the possibility of resistant insects over-wintering and contributing to the next season's target pest population.

Additionally, if possible, and prior to the application of alternative control measures or destruction of crop residue, Syngenta will collect samples of the insect population in the affected fields for laboratory rearing and testing. Such rearing and testing shall be conducted as expeditiously as practical.

Confirmed resistance

EPA defines *confirmed resistance* to mean, in the case of field reports of unexpected levels of damage from the key target pests, that all the following criteria are met:

- There is >30% insect survival and commensurate insect feeding in a bioassay, initiated with neonate larvae, that uses methods that are representative of exposure to *Bt* corn hybrids under field conditions (SWCB only).
- In standardized laboratory bioassays using diagnostic concentrations of the *Bt* protein suited to the target pest in question, the pest exhibits resistance that has a genetic basis and the level of survivorship indicates that there may be a resistance-allele frequency of ≥ 0.1 in the sampled population.
- In standardized laboratory bioassays, the LC_{50} exceeds the upper limit of the 95% confidence interval of the LC_{50} for susceptible populations surveyed both in the original baselines developed for this pest species and in previous years of field monitoring.

iii) Response to Confirmed Resistance in a Key Target Pest as the Cause of Unexpected Levels of Damage in the Field

When field resistance is *confirmed* (as defined above), the following steps will be taken by Syngenta:

- EPA will receive notification within 30 days of resistance confirmation;
- Affected customers and extension agents will be notified about confirmed resistance within 30 days;
- Monitoring will be increased in the affected area and local target pest populations will be sampled annually to determine the extent and impact of resistance;
- If appropriate (depending on the resistant pest species, the extent of resistance, the timing of resistance, and the nature of resistance, and the availability of suitable alternative control measures), alternative control measures will be employed to reduce or control target pest populations in the affected area. Alternative control measures may include advising customers and extension agents in the affected area to incorporate crop residues into the soil following harvest to minimize the possibility of over-wintering insects, and/or applications of chemical insecticides;
- Unless otherwise agreed with EPA, stop sale and distribution of the relevant lepidopteran-active *Bt* corn hybrids in the affected area immediately until an effective local mitigation plan approved by EPA has been implemented;
- Syngenta will develop a case-specific resistance management action plan within 90 days according to the characteristics of the resistance event and local agronomic needs. Syngenta will consult with appropriate stakeholders in the development of the action plan, and the details of such a plan shall be approved by EPA prior to implementation;
- Notify affected parties (e.g., growers, consultants, extension agents, seed distributors, university cooperators and state/federal authorities as appropriate) in the region of the resistance situation and approved action plan; and

- In subsequent growing seasons, maintain sales suspension and alternative resistance management strategies in the affected region(s) for the *Bt* corn hybrids that are affected by the resistant population until an EPA-approved local resistance management plan is in place to mitigate the resistance.

A report on results of resistance monitoring and investigations of damage reports must be submitted to the Agency annually by August 31st each year, beginning in 2010, for the duration of the conditional registration.

3) The Agency is imposing the following conditions for the mCry3A toxin expressed in MIR604 corn:

i) A revised mCry3A monitoring plan that incorporates *Bt*11 x MIR162 x MIR604 corn must be submitted to the Agency within 90 days of the date of registration. Syngenta must monitor for mCry3A resistance and/or trends in increased tolerance for corn rootworm. Sampling should be focused in those areas in which there is the highest risk of resistance development. In addition to mortality assays, consider utilizing sublethal bioassays (e.g., head capsule measurements) and molecular marker methods for corn rootworm monitoring.

ii) By January 31, 2010, submit data generated by the following actions: (a) initiate establishment of CRW strains that are resistant to mCry3A and investigate the nature, inheritance, and fitness costs of specific mechanisms of resistance to mCry3A, (b) study the behavioral deterrence (avoidance) mechanism further, and (c) continue studies on the biological impact of CRW adults surviving on corn expressing the mCry3A toxin.

iii) Syngenta must develop and validate an appropriate discriminating or diagnostic dose assay by January 31, 2010.

iv) Syngenta must finalize rootworm damage guidelines and submit these to EPA by January 31, 2010.

v) Syngenta must follow-up on grower, extension specialist or consultant reports of unexpected damage or control failures for corn rootworm.

vi) Syngenta must provide EPA with an annual resistance monitoring report by August 31st each year, beginning in 2010, reporting on populations collected the previous year.

vii) The following program summary describes, in order or events, the steps that must be taken to implement a remedial action plan if resistance to corn rootworm is confirmed (this general process has been implemented for other lepidopteran and corn rootworm *Bt* corn products).

1. *Definition of Suspected Resistance*. Resistance will be suspected if investigations of of unexpected damage reports show that:

   - implicated maize plant roots were expressing the mCry3A protein at the expected level;
   - alternative causes of damage or lodging, such as non-target pest insect species, weather, physical damage, larval movement from alternate hosts, planting errors, and other reasonable causes for the observations, have been ruled out;
   - the level of damage exceeds guidelines for expected damage.

   If resistance is "suspected," Syngenta will instruct affected growers to use alternate pest control measures such as adulticide treatment, crop rotation the following year, or use of soil or seed insecticides the following year. These measures are intended to reduce the possibility of potentially resistant insects contributing to the following year's pest population.

2. *Confirmation of Resistance.* Resistance will be confirmed if all of the following criteria are met by progeny from the target pest species sampled from the area of "suspected resistance":

   - the proportion of larvae that can feed and survive on mCry3A roots from neonate to adult is significantly higher than the baseline proportion (currently being established);
   - the LC_{50} of the test population exceeds the upper limit of the 95% confidence interval for the LC_{50} of a standard unselected population and/or survival in the diagnostic assay is significantly greater than that of a standard unselected population, as established by the ongoing baseline monitoring program;
   - the ability to survive is heritable;
   - mCry3A plant assays determine that damage caused by surviving insects would exceed economic thresholds; and
   - the identified frequency of field resistance could lead to widespread product failure if subsequent collections in the affected field area(s) demonstrated similar bioassay results.

3. *Response to Confirmed Resistance.* When resistance is "confirmed," the following steps will be taken:

   - EPA will receive notification within 30 days of resistance confirmation;
   - affected customers and extension agents will be notified about confirmed resistance;

- affected customers and extension agents will be encouraged to employ alternative corn rootworm control measures;
- sale and distribution of mCry3A corn in the affected area will cease immediately; and
- a long-term resistance management action plan will be devised according to the characteristics of the resistance event and local agronomic needs. [The details of such a plan should be approved by approved by EPA and all appropriate stakeholders.]

e) Annual Reporting Requirements for *Bt*11 x MIR162 x MIR604 Corn

1) Annual Sales: reported and summed by state (county level data available by request) January 31st each year, beginning in 2010;

2) Grower Agreements: number of units of Bt11 x MIR162 x MIR604 corn seed shipped or sold and not returned, and the number of such units that were sold to persons who have signed grower agreements, January 31st each year, beginning in 2010;

3) Grower Education: substantive changes to education program completed previous year, January 31st each year, beginning in 2010;

4) Compliance Assurance Program: compliance assurance program activities and results for the prior year and plans for the compliance assurance program for the current year, January 31st each year, beginning in 2010;

5) Compliance Survey Results: results of annual surveys for the prior year and survey plans for the current year; full report January 31st each year, beginning in 2010;

6) Insect Resistance Monitoring Results: results of monitoring and investigations of damage reports, August 31st each year, beginning in 2010.

IV. Regulatory Position for *Bacillus thuringiensis* (*Bt*) 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)

Pursuant to Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) section 3(c)(7)(C), the Environmental Protection Agency (EPA) may conditionally register a new pesticide active ingredient for a period of time reasonably sufficient for the generation and submission of required data that are lacking because insufficient time has elapsed since the imposition of the data requirement for those data to be developed. EPA may grant such conditional registration only if EPA determines that (1) the use of the pesticide product during the period of the conditional registration will not cause any unreasonable adverse effect on the environment, and (2) the registration and use of the pesticide during the conditional registration is in the public interest. EPA determines that all of these criteria have been fulfilled.

The first criterion under FIFRA section 3(c)(7)(C) mentioned above has been met because insufficient time has elapsed since the imposition of the data requirements for:

1) A validation of the analytical method performed by Syngenta (as described in Standard Operating Procedure 2.91) that provides 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.

2) A 7–14 day *Daphnia* study, as per the Office of Prevention, Pesticides, and Toxic Substances (OPPTS) 885.4240 guideline (Aquatic Invertebrate Testing), on Vip3Aa20. 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 *Daphnia* study.

3) Insect resistance management data for Vip3Aa20: (1) submission of a grower agreement, associated stewardship documents, and a written description of a system, which assures that growers will sign grower agreements and comply with the requirements of the insect resistance management program; (2) development of a compliance assurance program for refuge requirements; (3) generation of field efficacy and/or a protein expression report for Southwestern corn borer, which show that the stacked and/or pyramided products (i.e., *Bt*11 x MIR162 corn and *Bt*11 x MIR162 x MIR604 corn) have the same dose profile as their single-trait products; (4) development of baseline susceptibility and diagnostic concentration determinations for resistance monitoring of Southwestern corn borer and corn earworm; (5) development of a detailed resistance monitoring plan for the key target pests of Southwestern corn borer and corn earworm; and (6) completion of a final remedial action plan in the event of pest resistance.

The applicant submitted or cited data sufficient for EPA to determine that conditional registration of

Bacillus thuringiensis Vip3Aa20 insecticidal protein and the genetic material necessary for its production (*via* elements of vector pNOV1300) in Event MIR162 maize under FIFRA section 3(c)(7)(C) will not result in unreasonable adverse effects to the environment, as discussed above. The applicant submitted and/or cited satisfactory data pertaining to the proposed use. The human health effects data and non-target organism effects data are considered sufficient for the period of the conditional registration. These data demonstrate that no foreseeable human health hazards or ecological effects are likely to arise from the use of the product and that the risk of resistance developing to Vip3Aa20 insecticidal protein, during the time of the conditional registration, is not expected to be significant.

Registration of *Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production (*via* elements of vector pNOV1300) in Event MIR162 maize is in the public interest because:

1. 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 plant-incorporated protectants (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.

2. Vip3Aa20 has a novel 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 MIR162 maize, *Bt*11 x MIR162 corn, *Bt*11 x MIR162 x MIR604 corn, and other corn PIP products.

3. The availability of multiple *Bt* corn products, created by combination of the MIR162 trait with other registered PIPs, will increase grower choice and price competition, likely resulting in lower seed prices for consumers and higher adoption rates.

4. Registration of MIR162 maize, *Bt*11 x MIR162 corn, and *Bt*11 x MIR162 x MIR604 corn is expected to result in further reduction of chemical insecticide use by corn growers. Lower insecticide use should result in benefits for both human health and the environment.

In view of these minimal risks and the clear benefits related to *Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production (*via* elements of vector pNOV1300) in MIR162 maize, EPA believes that the use of the product (and its associated stacked and/or pyramided products) during the limited period of the conditional registration will not cause any unreasonable adverse effects.

Although the data with respect to this particular new active ingredient are satisfactory, they are not sufficient to support an unconditional registration under FIFRA section 3(c)(5). Additional data are necessary to evaluate the risk posed by the continued use of products associated with this new active ingredient. Consequently, EPA is imposing the data requirements specified earlier in part III.

EPA has determined, as explained in the Benefits and EPA Public Interest Finding chapter of this Biopesticides Registration Action Document (BRAD), that the third criterion for a FIFRA section 3(c)(7)(C) conditional registration has been fulfilled because the use of *Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production (*via* elements of vector pNOV1300) in MIR162 maize under this registration is in the public interest.

The data submitted in support of this registration under Section 3(c)(7)(C) of the Federal Insecticide, Fungicide, and Rodenticide Act have been reviewed and determined to be adequate. Studies mentioned above are included in the terms, conditions, and limitations of this registration. This registration will not cause unreasonable adverse effects to man or the environment and is in the public interest.

The expiration date of the registration has been set to December 31, 2011.