

**BIOPESTICIDES REGISTRATION ACTION DOCUMENT**

*Bacillus thuringiensis* modified Cry1Ab (SYN-IR67B-1) and Vip3Aa19 (SYN-IR102-7) insecticidal proteins and the genetic material necessary for their production in COT102 X COT67B cotton

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

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### Regulatory Action Team

#### **Product Characterization and Human Health**

John Kough, Ph.D.  
Annabel Waggoner, B.S.  
Rebecca Edelstein, Ph.D.  
Sharlene Matten, Ph.D.  
Chris Wozniak, Ph.D.

#### **Environmental Fate and Effects**

Zigfridas Vaituzis, Ph.D.  
Annabel Waggoner, B.S.

Tessa Milofsky, M.S.  
Mika Hunter, B.S.

**Insect Resistance Management**

Jeannette Martinez, M.S.

**Benefit Assessment**

Jeannette Martinez, M.S.

**Biopesticides Registration Action Document Team Leader**

Alan Reynolds, M.S.

**Office of General Council**

Angela Huskey, Esq.  
Chris Kaczmarek, Esq.  
Keith Matthews, Esq.

## I. Overview

### A. Executive Summary

EPA has conditionally registered a new pesticide product containing Syngenta Seeds Inc.'s new active ingredients, *Bacillus thuringiensis* Vip3Aa19 (OECD Unique Identifier SYN-IR102-7) and modified Cry1Ab (OECD Unique Identifier SYN-IR67B-1) insecticidal proteins and the genetic material necessary for their production in COT102 X COT67B cotton. Syngenta has trademarked this product as VipCot -- the trademark name of VipCot will be used in this document to describe COT102 X COT67B cotton. The Agency has determined that the use of this pesticide is in the public interest and that it will not cause any unreasonable adverse effects on the environment during the time of conditional registration.

The new cotton plant-incorporated protectant, VipCot, produces its own insecticidal proteins within the cotton plant. These proteins were derived from *Bacillus thuringiensis* (Bt), a naturally occurring soil bacterium. The modified Cry1Ab and Vip3Aa19 proteins used in this product control lepidopteran pests of cotton.

On June 26, 2008, tolerance exemptions under 40 CFR Part 174 were approved for *Bacillus thuringiensis* modified Cry1Ab protein as identified under OECD Unique Identifier SYN-IR67B-1 in cotton (40 CFR 174.529) and Vip3Aa proteins in corn and cotton (40 CFR 174.501). The exemption for Vip3Aa is inclusive of the Vip3Aa19 protein and its use in cotton.

#### Benefits

Results of efficacy trials conducted in 2005 and 2006 show that VipCot cotton and its single event cotton isolines provide good protection against three major cotton pests: tobacco budworm (*Heliothis virescens*), cotton bollworm (*Helicoverpa zea*), and pink bollworm (*Pectinophora gossypiella*). The Vip3Aa19 protein expressed in VipCot cotton has not been previously registered and provides a unique mode of action. When coupled with modified Cry1Ab in VipCot, the proteins have the potential to provide benefits for insect resistance management including: high-dose (for both proteins expressed together) against the major target pests, lack of cross-resistance (Vip3Aa19), and the potential to delay development of resistance in other cotton varieties expressing Cry toxins. As an additional registered Bt cotton product, VipCot will likely result in direct and indirect human and environmental health benefits by providing growers with an additional choice of Bt cotton option and the potential to increase grower choice and price competition, resulting in lower seed prices for consumers and higher adoption rates. Registration of VipCot 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 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 Fed. Reg. 7628 (*Conditional Registration of New Pesticides*, March 5 1986). On the basis of analysis utilizing these criteria, EPA concludes that the use of VipCot protected cotton will be in the public interest, because it results in direct and indirect human and environmental health benefits by providing growers with an additional Bt cotton product which has the potential to extend the useful life of Bt cotton technology generally due to a novel mode of action (Vip3Aa19) and low likelihood of cross-resistance with other Bt Cry proteins.

### Product Characterization

VipCot (COT102 x COT67B) was developed by conventional breeding of COT102 (Vip3Aa19) plants with COT67B (modified Cry1Ab) plants.

Event COT102 cotton, which was developed by *Agrobacterium*-mediated transformation of cotton using elements of a vector referred to as both pNOV3001 and pCOT1, expresses the insecticidal protein, Vip3Aa19 as well as a selectable marker, hygromycin B phosphotransferase (APH4). The Vip3Aa19 protein is intended to control several lepidopteran pests of cotton including *Heliothis virescens* (tobacco budworm, TBW), *Helicoverpa zea* (cotton bollworm, CBW), *Spodoptera frugiperda* (fall armyworm), *Spodoptera exigua* (beet armyworm), and *Trichoplusia ni* (cabbage looper). Vip3A is a vegetative (i.e., produced during the vegetative stage of bacterial growth) insecticidal protein from *Bacillus thuringiensis* (*Bt*), a gram positive bacterium commonly found in soil.

Event COT67B cotton, which was developed by *Agrobacterium*-mediated transformation of cotton using elements of vectors pNOV4641 and pNOV1914, expresses the insecticidal protein, modified Cry1Ab. This protein contains an additional 26 amino acid sequence at the C-terminus (termed the 'Geiser motif'). The modified Cry1Ab protein is intended to control several lepidopteran pests of cotton including *Heliothis virescens* (tobacco budworm), *Helicoverpa zea* (cotton bollworm), *Pectinophora gossypiella* (pink bollworm), *Spodoptera frugiperda* (fall armyworm), *Spodoptera exigua* (beet armyworm), and *Trichoplusia ni* (cabbage looper).

DNA characterization (i.e., Southern blot analysis) was used to confirm the integrity of the COT102 and COT67B inserts in the stacked product COT102 x COT67B. Samples from COT102 x COT67B cotton gave the same results as those observed for the individual events, indicating that the molecular characterization data provided for the individual events are also applicable to COT102 x COT67B.

Protein expression data, together with data indicating that there is no evidence of either a synergistic or antagonistic interaction between Vip3Aa19 and modified Cry1Ab in cotton bollworm or tobacco budworm, demonstrate that data on the individual events and individual proteins can be used to support the safety of the COT102 x COT67B (VipCot) combined product.

#### Human Health Assessment

There is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the modified Cry1Ab and Vip3Aa19 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 no toxicity to mammals has been observed, nor any indication of allergenicity potential for the plant-incorporated protectant.

Syngenta submitted four acute oral toxicity studies conducted on mice, which all indicated that Vip3Aa is 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 transgenic corn leaf tissue as the test material. No treatment-related adverse effects were observed in any of the studies. The oral LD<sub>50</sub> for mice (males, females, and combined) was greater than 3675 mg Vip3Aa/kg body weight (the highest dose tested). For modified Cry1Ab, an acute oral toxicity study in mice indicated that the protein is non-toxic to humans. Groups of five male and five female mice were given 0 or 1830 mg/kg bodyweight microbially-produced modified Cry1Ab by oral gavage as a single dose. There were no effects on clinical condition, body weight, food consumption, clinical pathology, organ weight, or macroscopic or microscopic pathology that were attributed to the test substance.

Since Vip3Aa and modified Cry1Ab 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 and modified Cry1Ab 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 Vip3Aa19 and modified Cry1Ab 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 and modified Cry1Ab proteins were digested rapidly in simulated

gastric fluid containing pepsin.

4. Glycosylation. Vip3Aa and modified Cry1Ab (expressed in cotton) were shown not to be glycosylated.
5. Conclusion. Considering all of the available information, EPA has concluded that the potential for Vip3Aa and modified Cry1Ab to be food allergens is minimal.

#### Environmental Assessment

The Agency concludes that for the VipCot cotton breeding stack (COT102 x COT67B, containing modified Cry1Ab and Vip3Aa19) no unreasonable adverse effects will result to the environment or any federally-listed threatened or endangered species from commercial cultivation of COT102 x COT67B cotton. This conclusion is based on prior assessments conducted on Vip3Aa and Cry1Ab proteins individually. Furthermore, the Agency has determined that Events COT102, COT67B, and VipCot cotton will have No Effect (NE) on endangered and/or threatened species listed by the US 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 VipCot cotton may result in fewer adverse impacts to non-target organisms than result from the use of chemical pesticides. Under normal circumstances, Bt cotton 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 controls (IPM) for secondary pests such as aphids and leafhoppers. Therefore, the overall result of cultivation of VipCot cotton, expressing Vip3Aa19 and modified Cry1Ab proteins, is that the number of chemical insecticide applications for non-target pest control will be reduced for management of multiple pest problems.

#### Insect Resistance Management

In order to reduce the possibility of the target pests developing resistance to Vip3Aa19 and modified Cry1Ab (as expressed in VipCot cotton), EPA is requiring Syngenta Seeds, Inc. to ensure that a portion of the planted acreage of this product be set aside where non-Bt cotton will be grown to serve as a "refuge." Under the established refuge strategy for Bt cotton, growers can choose from three structured refuge options:

Option 1: 95:5 external structured, unsprayed refuge; 150 ft wide, within ½ mile of edge of field.

Option 2: 80:20 external sprayed refuge; within 1 linear mile, preferably ½ mile, of edge of field.

Option 3: 95:5 embedded refuge; contiguous or within 1 mile<sup>2</sup> of field and 150 ft wide.

In addition to the refuge options above, growers of VipCot may participate in a community refuge plan in which multiple growers contribute to the overall required refuge acres by planting 20% external, sprayed or 5% external, unsprayed refuge.

BPPD has concluded that based on the modeling, dose, and efficacy studies, the requested refuge options 1-3 and community refuge plan are acceptable for VipCot cotton. Syngenta will also be required to develop and conduct a resistance monitoring program for Vip3Aa19 and modified Cry1Ab with the major target pests (cotton bollworm, tobacco budworm, and pink bollworm). Additional requirements for remedial action (in the event of resistance), grower education, compliance assurance, and annual reported will also be implemented for VipCot as terms of registration.

## **B. Use Profile**

**Pesticide Name:** *Bacillus thuringiensis* Vip3Aa19 (OECD Unique Identifier SYN-IR102-7) and modified Cry1Ab (OECD Unique Identifier SYN-IR67B-1) insecticidal proteins and the genetic material necessary for their production in COT102 X COT67B cotton

**Trade and Other Names:** VipCot Cotton; COT102 X COT67B Cotton

**OPP Chemical Code:** 006499 (Vip3Aa19) and 006529 (modified Cry1Ab)

**Basic Manufacturers:** Syngenta Seeds, Inc.

**Type of Pesticide:** Plant-Incorporated Protectant

**Uses:** Cotton

**Target Pest(s):** tobacco budworm, cotton bollworm, pink bollworm

## **C. Regulatory History**

Syngenta Seeds, Inc. was issued an Experimental Use Permit (EUP) for VipCot Bt cotton containing Vip3Aa19 (Event COT102) and modified Cry1Ab (Event COT67B) on April 26, 2007 (EPA Reg. No. 67979-EUP-7). These proteins were selected to provide protection of cotton from feeding damage caused by major lepidopteran pests including tobacco budworm, cotton bollworm, and pink bollworm. On April 26, 2007, EPA established a temporary exemption from the requirement of a tolerance for Vip3Aa19 (72 FR 26300, amended 72 CFR 40752; 40 CFR 174.501) in the food and

feed commodities of cotton. For the purpose of the EUP, modified Cry1Ab was determined to be covered under the permanent tolerance exemption for Cry1Ab in all crops (40 CFR 174.511). Both the EUP and temporary tolerance exemption were originally set to expire on May 1, 2008. However, Syngenta was granted an extension of both the EUP and temporary tolerance exemption (72 FR 68744) on November 27, 2007 which expire on May 1, 2009.

A separate EUP (EPA Reg. No. 67979-EUP-5) was previously issued to Syngenta for two Bt cotton events (Event COT202 and COT203) containing Vip3A. These two events were not part of the more recent VipCot EUP and have not been proposed for commercial registration. This EUP expired on March 31, 2006.

On December 14, 2006, Syngenta submitted an application (EPA Reg. No. 67979-O) to register VipCot (Event COT 102 x Event COT67B) under Section 3 of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). On April 5, 2007, Syngenta submitted a second application for a seed increase registration (EPA Reg. No. 67979-RR). This application was subsequently withdrawn by the registrant on January 7, 2008.

On September 6, 2007, Syngenta submitted a petition to EPA under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended by the Food Quality Protection Act of 1996 (FQPA), requesting a permanent tolerance exemption for Vip3Aa in all plants (PP 7F7254). A separate petition was submitted to request a permanent exemption for modified Cry1Ab in all plants on November 9, 2007 (PP 7F7290). After review of the supporting data, EPA determined that the permanent tolerance exemptions would be limited to corn and cotton (Vip3Aa) and cotton (modified Cry1Ab).

On June 26, 2008 (73 FR 45620 and 73 FR 40760), the Agency established permanent exemptions from the requirement of a tolerance for residues of the *Bacillus thuringiensis* Vip3Aa proteins in corn and cotton (40 CFR 174.501) and modified Cry1Ab protein as identified under OECD Unique Identifier SYN-IR67B-1 in cotton (40 CFR 174.529) when used as plant-incorporated protectants.

On June 26, 2008, a conditional registration was issued for VipCot Bt Cotton (EPA Reg. No. 67979-9).

## II. Science Assessment

The classifications that are found for each data submission are assigned by 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,” EPA 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 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.

### II.A. Product Characterization

#### II.A.1. Event COT102 Cotton (OECD Unique Identifier: SYN-IR102-7) Expressing Vip3Aa19

Event COT102 cotton, which was developed by *Agrobacterium*-mediated transformation of cotton using elements of a vector referred to as both pNOV3001 and pCOT1, expresses the insecticidal protein, Vip3Aa19 as well as a selectable marker, hygromycin B phosphotransferase (APH4). The Vip3Aa19 protein is intended to control several lepidopteran pests of cotton including, but not limited to, *Helicoverpa zea* (cotton bollworm/corn earworm), *Heliothis virescens* (tobacco budworm), *Spodoptera frugiperda* (fall armyworm), *Spodoptera exigua* (beet armyworm), and *Trichoplusia ni* (cabbage looper). Vip3A is a vegetative (i.e., produced during the vegetative stage of bacterial growth) insecticidal protein from *Bacillus thuringiensis* (*Bt*), a gram positive bacterium commonly found in soil.

#### Transformation System:

COT102 cotton was produced by *Agrobacterium tumefaciens*-mediated transformation of hypocotyls of *Gossypium hirsutum* L. cultivar Coker 312 with plasmid pNOV3001 (also referred to as pCOT1). Plasmid pNOV3001 (pCOT1) contains T-DNA with the *vip3Aa19* and *aph4* expression cassettes. The *vip3Aa19* expression cassette contains the *vip3Aa19* coding sequence under the regulation of the Act2 promoter and intron (derived from *Arabidopsis thaliana*), and NOS terminator (derived from *Agrobacterium tumefaciens*). The *aph4* expression cassette contains the *aph4* coding sequence under the regulation of the Ubq3 promoter and intron (derived from *Arabidopsis thaliana*) and the NOS terminator (derived from *Agrobacterium tumefaciens*). The *vip3Aa19* gene encodes a protein that differs from the Vip3Aa1 protein from *Bacillus thuringiensis* strain AB88 by one amino acid at position 284 (The *vip3Aa1* gene encodes lysine at position 284, and the *vip3Aa19* gene encodes glutamine). Vip3Aa19 confers resistance to several lepidopteran pests. The *aph4* gene encodes

hygromycin B phosphotransferase (APH4), an enzyme that catalyzes the phosphorylation of hygromycin and some related aminoglycosides. Expression of APH4 allows growth in the presence of hygromycin and was used as a selectable marker, enabling selection of transformed cells.

**Characterization of the DNA Inserted in the Plant and Inheritance and Stability:**

Characterization of the DNA isolated from event COT102 cotton using restriction enzyme digests and Southern blot analysis as well as DNA sequencing indicates that the DNA was inserted in the cotton genome at a single locus, and the insert contains one copy each of the *vip3Aa19* and *aph4* expression cassettes. There were no other detectable elements other than those associated with the respective cassettes. No backbone sequences from plasmid pNOV3001 (pCOT1) were detected in the cotton genome. Southern blot analysis and protein expression data also demonstrated the stability of the insert over multiple generations.

**Protein Characterization:**

The insecticidal protein produced in event COT102 cotton, designated as Vip3Aa19<sup>a</sup>, is a variant of the naturally occurring Vip3Aa1 protein isolated from *Bacillus thuringiensis* strain AB88, differing from the Vip3Aa1 protein by one amino acid (Vip3Aa19 contains a glutamine at position 284, while Vip3Aa1 contains a lysine). Both proteins are 789 amino acids in length and have a molecular weight of approximately 89 kDa. Syngenta has also developed a transgenic corn variety, MIR162, that produces another variant, designated as Vip3Aa20, differing from the naturally occurring Vip3Aa1 protein by two amino acids; at position 284, Vip3Aa20 has the same amino acid substitution as Vip3Aa19 (i.e., K284Q), and in addition, at position 129, Vip3Aa20 contains an isoleucine, while Vip3Aa1 contains a methionine (M129I).

The following techniques were used to characterize and compare the plant-produced and the *E. coli*-produced Vip3Aa proteins: sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blot analysis, densitometry, mass spectrometry, glycosylation analysis, N-terminal amino acid sequencing, and insecticidal activity assays. Glycosylation analysis indicated that the proteins are not glycosylated. These analyses demonstrated the structural and functional similarity between the plant-produced Vip3Aa19 and the *E. coli*-produced Vip3Aa19, Vip3Aa20, and Vip3Aa1 proteins and justified the use of *E. coli*-produced proteins in toxicity studies.

**Analytical Detection Methods:**

Syngenta has provided a validation study for SeedChek Vip3A/FLCry1Ab, a lateral flow test kit that detects both Vip3A and Cry1Ab. The SeedChek Vip3A/FLCry1Ab lateral flow test kit was tested for the qualitative detection of modified Cry1Ab and Vip3A proteins in cotton seed and cotton leaf. The study showed that the SeedChek kit is able to detect Vip3A and Cry1Ab in both cotton seed and

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<sup>a</sup> Prior to receiving the Crickmore designation of Vip3Aa19, the protein produced in COT102 was referred to as Vip3A or Vip3Aa.

cotton leaf. No unexpected cross reactivity with other transgenic varieties or nontransgenic controls was observed. An independent lab validation of this method is still needed.

**Protein Expression:**

Expression level data were provided for Vip3Aa19 and APH4 in different plant tissues and at different growth stages in COT102.

**Table 1.** Mean Expression Levels of Vip3Aa19 and APH4 from COT102 Plant Tissues

Tissue Type	Vip3Aa19 ( $\mu\text{g/g}$ dry weight $\pm$ standard deviation)	APH4 ( $\mu\text{g/g}$ dry weight $\pm$ standard deviation)
Leaves*	44 $\pm$ 10 - 277 $\pm$ 41	< 0.42 - 8.2 $\pm$ 1.4
Squares	116 $\pm$ 22	2.2 $\pm$ 0.4
Flowers	162	1.68
Pollen	3.47	64.3
Bolls	19 $\pm$ 4	< 0.39
Whole Plants	25 $\pm$ 4	< 0.37
Seed	7 $\pm$ 2	1.4 $\pm$ 0.3
Roots	16 $\pm$ 2	0.53 $\pm$ 0.11

\*Ranges reflect means at different growth stages for leaves

The data submitted for product characterization for event COT102 cotton are summarized in Table 2 below.

**Table 2.** Product Characterization Data Submitted for Event COT102 Cotton (reviewed in Edelstein 2008 unless otherwise noted)

Study Type/Title	Summary	MRID #
Expression Levels/ Quantitation of VIP3A and APH4 Protein in Cotton Tissues and Whole Plants Derived from Transformation Event COT102 <sup>b</sup>	Transgenic cotton plants (COT102) and a non-transgenic isolate (Coker 312) were grown concurrently in 2001 in Camilla, GA; Maricopa, AZ; and Idalou, TX. Ten whole transgenic plants (including roots) and two control plants were harvested approximately 2, 4, 9, 13, 15, and 22 week post-emergence (stages: four-leaf, squaring, first white bloom, peak bloom, first open boll, pre-harvest, respectively). Tissue extracts were analyzed for VIP3A and APH4 by ELISA. VIP3A protein was detected in COT102 whole plants, leaves, roots, squares, and bolls at all six developmental stages examined. VIP3A levels varied in all plant tissues, generally declined with time, but stayed constant in the roots. The highest levels were found in	45835801

<sup>b</sup> Study submitted with EUP request and reviewed in memorandum from C. Wozniak to L. Cole dated March 24, 2004.

Study Type/Title	Summary	MRID #
	<p>leaves at the squaring stage (mean of 8.56 to 10.78 µg VIP3A/g fresh tissue). Low VIP3A levels were found in seed (mean of 2.51 to 3.23 µg VIP3A/g) and in pollen (1.09 µg VIP3A/g). VIP3A was not detected in cotton fiber or nectar. The protein marker, APH4, was detected in COT102 plants at low, non-quantifiable levels at some developmental stages in leaves, roots, bolls, squares, and whole plants and at quantifiable levels in pollen (2.25 µg APH4/g air-dried pollen). APH4 was not detected in cotton fiber or nectar. Geographic location appeared not to have a significant effect on VIP3A levels, but no statistical analysis was done. APH4 levels appears to be similar across locations, but the lack of data points in many instances and the detectable levels falling below the level of quantitation (LOQ) do not allow for any definitive conclusions to be made. The estimated amount of VIP3A/acre cotton varied considerably among the developmental stages with the greatest mean level found at the peak bloom stage (105.80 g VIP3A/acre based on whole plant VIP3A levels).</p> <p><b>Classification: ACCEPTABLE</b></p>	
<p>Characterization of Inserted DNA/Molecular Characterization and Genetic Stability of Event COT102<sup>b</sup></p>	<p>Southern blot analysis and DNA sequencing suggest that event COT102 has one transgene insertion site with a single copy of intact <i>vip3A(a)</i> and <i>aph4</i> expression cassettes (containing one copy of the <i>vip3A(a)</i> gene, <i>aph4</i> gene, actin-2 promoter, and ubq3 promoter). DNA sequence alignment revealed an exact sequence match between the pCOT-1 vector and event COT102, and showed the lack of <i>Agrobacterium</i> sequence beyond the T-DNA borders. VIP3 protein expression measurement (by ELISA) of five generations of COT102 seedlings (F1, BC1F2, BC2F1, BC2F2, and BC3F1) showed that the <i>vip3A(a)</i> gene was stable across generations and segregated in a Mendelian fashion, consistent with a single transgene insertion site. MRID 458358-02 provided very scant experimental details. Insufficient experimental methods details were provided for the Southern blots, DNA cloning and sequencing, PCR analysis, and protein detection and segregation analysis by ELISA, precluding confirmation of their appropriateness by an independent reviewer. Sample Southern blots demonstrating the integration copy number and lack of rearrangements through appropriate restriction analyses must be provided in order to assess the results of this study. Further information is required regarding the number of plants utilized in the segregation and heritability analysis.</p> <p><b>Classification: SUPPLEMENTAL</b>, upgradeable to acceptable pending submission of additional methods details and correction/clarification of typographic errors in Figure 1, Figure 2, and/or the text of MRID 458358-02.</p> <p><b>Superseded by MRID 47017603</b></p>	<p>45835802</p>
<p>Characteristics of <i>Bacillus thuringiensis</i> VIP3A Protein and VIP3A Cotton Plants Derived from Event COT102<sup>b</sup></p>	<p>The <i>Bacillus thuringiensis</i> (Bt) 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 <i>Bacillus thuringiensis</i> VIP3A Protein and the genetic material necessary for its production (pCOT1 in cotton). The product also contains ≤0.0001% dry</p>	<p>45766501</p>



















































































































































































































































