USDA/APHIS Environmental Assessment

Syngenta Petition 03-155-01p for Determination of Nonregulated
Status for Lepidopteran Resistant Event COT102

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture, has prepared an environmental assessment in response to a petition (APHIS Number 03-155-01p) received from Syngenta seeking a determination of nonregulated status for their genetically engineered cotton designated Event COT102 (OECD unique identifier SYN-IR102-7) under APHIS regulations at 7 CFR Part 340. The plants have been engineered with a gene that confers resistance to certain lepidopteran insects.

U.S. Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 03-155-01p) from Syngenta (Research Park Triangle, NC) seeking a determination of nonregulated status for their transgenic VIP3A lepidopteran insect resistant cotton designated as Event COT102 (OECD unique identifier SYN-IR102-7). Syngenta seeks a determination that Event COT102 and its progeny do not present a plant pest risk and, therefore, are no longer regulated articles under regulations at 7 CFR Part 340.

Event COT102 was engineered to be lepidopteran resistant by inserting a gene for a Vegetative Insecticidal Protein (VIP3A) into the cotton genome. The gene originates from the common soil bacterium *Bacillus thuringiensis* strain AB88, and was introduced into these cotton plants via an *Agrobacterium*-mediated transformation protocol. A second gene used during the development process was also introduced into Event COT102 that encodes for a selectable marker (APH4).

This EA specifically addresses the potential for impacts to the human environment through the use in agriculture of Event COT102. The United States Environmental Protection Agency (EPA) has authority over the use in the environment of all pesticidal substances, including genetically engineered insecticides, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The Food and Drug Administration (FDA) has authority over food and feed issues of all genetically improved plants used as food or feed.

Field trials of Event COT102 cotton have been conducted under APHIS notification procedures (7 CFR 340.3). In accordance with APHIS procedures for implementing the National Environmental Policy Act (NEPA) (7 CFR 372), this EA has been prepared prior to issuing a determination of nonregulated status for Event COT102 cotton in order to specifically address the potential for impact to the environment through unconfined cultivation and use of the regulated articles in agriculture.

II. INTRODUCTION

A. Development of Event COT102

Syngenta has submitted a "Petition for Determination of Nonregulated Status" to the USDA, APHIS (APHIS number 03-155-01p) for genetically engineered cotton that is resistant to certain lepidopteran insects. The management of insects in cotton fields can be an expensive and in the absence of biotechnology-derived products, often results in the applications of chemical insecticides. Globally, twenty five percent of all pesticides are applied to cotton. As a consequence of the use of genetically engineered cotton, the number of applications and amount of chemical insecticide has been reduced (http://www.talksoy.com/ComparativeStudy/default.htm, http://www.ers.usda.gov/publications/aer810/). As an alternative to application of insecticides, farmers have the option of using cotton varieties expressing the Cry protein. Concern that resistance to Cry may build up in insect populations has fostered an interest in developing novel proteins with altered mode of action, such as the VIP3A protein in Event COT102. The rational for development of Event COT102 is to reduce the likelihood of build up of insect resistance to Bt-cotton and offer growers a novel option for insect control.

These cotton plants were genetically engineered to be insect resistant by inserting a gene from *Bacillus thuringiensis* strain AB88 that codes for the Vegetative Insecticidal Protein (VIP3A) into the cotton genome. This gene, along with its regulatory sequences, was introduced into these cotton plants via an *Agrobacterium*-mediated transformation protocol. This is a well characterized procedure that has been used widely for over a decade for introducing various genes of interest directly into plant genomes (Howard, *et al.* 1990). Event COT102 also contain a selectable marker gene used in the development of this cotton line.

APHIS authorized the first field testing of these cotton plants starting in 2000 and they have been field tested in the United States under the APHIS authorization numbers noted in Appendix D. Event COT102 cotton plants have been evaluated extensively to confirm that they exhibit the desired agronomic characteristics and do not present a plant pest risk. The field tests have been conducted in agricultural settings under physical and reproductive confinement conditions.

B. APHIS Regulatory Authority

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Plant Protection Act (7 U.S.C. 7701-7772) regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. These cotton plants have been considered regulated articles because they contain non-coding DNA regulatory sequences derived from plant pathogens and the vector agent used to deliver the transforming DNA is a plant pathogen.

Section 340.6 of the regulations, entitled "Petition for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk, and therefore should no longer be regulated. If APHIS determines that the regulated article is unlikely to present a greater plant pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. In such a case, APHIS authorizations (i.e., permits or notifications) would no longer be required for field testing, importation, or interstate movement of the nonregulated article or its progeny.

C. U.S. Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) Regulatory Authority

This genetically engineered cotton plant for use in plant resistance to certain lepidopteran pests are also subject to regulation by other U.S. government agencies. The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended (7 U.S.C. 136 *et seq*.). FIFRA requires that all pesticides, including herbicides, be registered prior to distribution or sale, unless exempt by EPA regulation. Under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended (21 U.S.C. 301 *et seq*.), pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance

or exemption from tolerance has been established. Residue tolerances for pesticides are established by EPA under the FFDCA, and the FDA enforces the tolerances set by the EPA. Syngenta has submitted a pesticide registration to EPA. EPA has issued a time-limited tolerance exception for the VIP3A protein which expires on May 1, 2005 and a tolerance exception for the APH4 selectable marker protein.

The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food. Syngenta submitted a food and feed safety and nutritional assessment summary for Event COT102. A final FDA decision is pending.

III. PURPOSE AND NEED

APHIS has prepared this EA before making a determination on the status of Event COT102 plants as regulated articles under APHIS regulations. The developer of these cotton plants, Syngenta, submitted a petition to USDA, APHIS requesting that APHIS make a determination that these cotton plants shall no longer be considered regulated articles under 7 CFR Part 340.

This EA was prepared in compliance with the National Environmental Policy Act (NEPA) of 1969 as amended, (42 USC 4321 *et seq*.) and the pursuant implementing regulations (40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372).

IV. ALTERNATIVES

A. No Action: Continuation as a Regulated Article

Under the Federal "no action" alternative, APHIS would not come to a determination that these cotton plants are not regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of Event COT102 lines of insect resistant cotton plants. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of insect resistant cotton plants.

B. Determination that Event COT102 Cotton is No Longer Regulated Articles, in Whole

Under this alternative, these insect resistant cotton plants would no longer be regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of insect resistant cotton plants. A basis for this determination would include a "Finding of No Significant Impact" under the National Environmental Policy Act of 1969, as amended (42 USC 4321 *et seq*.; 40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 342).

C. Determination that Event COT102 Cotton is No Longer Regulated Articles, in Part

The regulations at 7 CFR Part 340.6 (d) (3) (I) state that APHIS may "approve the petition in whole or in part."

APHIS could determine that the regulated article poses no significant risk in certain geographic areas, but may pose a significant risk in others. In such a case, APHIS might choose to approve the petition with a geographic limitation stipulating that the approved line could only be grown without APHIS authorization in certain geographic areas.

V. POTENTIAL ENVIRONMENTAL IMPACTS

Potential impacts to be addressed in this EA are those that pertain to the use of Event COT102 and its progeny in the absence of confinement.

A. Alternative A: No Action

If APHIS takes no action, commercial scale production of Event COT102 and its progeny is effectively precluded. These plants could still be grown in field trials for variety development as they have been for the past several years under APHIS authorizations (notifications). APHIS has evaluated field trial data reports submitted on Event COT102 and its progeny and noted no significant adverse effects on non-target organisms, no increase in fitness and no effect on the health of other plants. The Agency expects that future field tests would perform similarly.

With respect to commercial production, if APHIS were to take no action, cotton growers would still have the same options available to them for insect control as they currently have. USDA/NASS statistics collected from cotton growers most recently in 2000 (http://usda.mannlib.cornell.edu/reports/nassr/other/pcu-bb/agcs0501.txt) document significant use of 13 insecticides used in cotton fields in the 11 states surveyed. Planted area in the primary cotton producing states covered 14.4 million acres. The insecticides are listed in the following table taken from USDA/NASS statistics (http://usda.mannlib.cornell.edu/reports/nassr/other/pcu-bb/agcs0501.txt).

Upland Cotton: Agricultural Chemical Applications, States Surveyed, 2000*

Agricultural Chemical	Area Applied	Applications	Rate per Application	Rate per Crop Year	Total Applied
	Percent**	Number	Pounds per Acre		1,000 lbs
Insecticides:					
Acephate	12	1.5	0.50	0.76	1,288
Aldicarb	26	1.0	0.63	0.65	2,483
Carbofuran	5	1.0	0.24	0.25	172
Chlorpyrifos	5	1.5	0.63	1.01	659
Cyfluthrin	8	1.3	0.08	0.11	122
Cypermethrin	8	1.1	0.06	0.07	79
Dicrotophos	8	1.4	0.22	0.32	364
Lambda-cyhalothrin	9	1.5	0.02	0.03	46
Malathion	36	6.9	0.89	6.17	31,923
Methyl parathion	5	2.1	0.58	1.25	815
Oxamyl	11	1.8	0.25	0.47	722
Phorate	5	1.0	0.63	0.63	439
Spinosad	5	1.9	0.07	0.13	90

* States included are AL, AZ, AR, CA, GA, LA, MS, MO, NC, TN and TX.
** Percent listed are chemicals with application rates of greater than or equal to 5 percent of area applied.

On a global basis twenty five percent of the chemical pesticides are applied to cotton. Documented insecticidal application in the U.S. in 2000 totaled over 39 million pounds. The major chemical insecticides that are applied to crops are broad spectrum. These are significantly disruptive to many beneficial insects and have significant residue problems (http://www.talksoy.com/ComparativeStudy/default.htm). It is likely that the approximate rate of insecticidal application would continue without alternatives. Genetically engineered cotton will present growers with an alternative to use of broad spectrum insecticides. Growers in the U.S. and worldwide are experiencing significant reduction of insecticide application as the result of genetically engineered cotton containing Bt proteins (http://www.ers.usda.gov/publications/aer810/). Syngenta submitted documentation of reduction of insecticide application (petition Table 9.2) subsequent to the introduction of Bt-cotton products. Concern has been raised of a potential build up of Bt resistance in insect populations. VIP3A operates by a different mechanism of action compared to Cry products. Thus, VIP3A may aid in preventing the build up of Cry insect resistance and provides growers with an alternative to Cry derived protection.

B. Alternative B: Approval of the petition in Whole

If APHIS were to grant the petition for nonregulated status in whole, cotton Event COT102 and its progeny would no longer be considered regulated articles. APHIS' assessments of the environmental impacts are discussed in the following sections.

Plant pathogenic properties
APHIS considered the potential for the transformation process, the introduced DNA sequences and their expression products to cause or aggravate disease symptoms in cotton Event COT102 and its progeny or in other plants. We also considered whether data indicate that unanticipated unintended effects would arise from engineering of these plants. APHIS considered information from the scientific literature as well as data provided by the developer when conducting their field trials. A summary of the data that APHIS evaluated is contained in Appendix D of this Environmental Assessment.

Recipient organism
The plant material used for development of Event COT102 was Coker 312. Coker 312 was developed from a cross of Coker 100 x D&DP-15 and selected through successive generations of line selection. Cotton is not listed as a Federal Noxious weed or on these other weed lists (http://www.aphis.usda.gov/ppq/weeds/noxwdsa.html, http://www.extendinc.com/weedfreefeed/list-b.htm, http://www.weedawareness.org/weed%20list.html, http://edis.ifas.ufl.edu/TOPIC_BOOK_Florida_Weeds.

Transformation system
Event COT102 was developed using a disarmed *Agrobacterium*-mediated transformation system of sterile cotton hypocotyl tissue. Hygromycin was used to select for transformed plant cells that contained the *aph4* gene and linked *vip3A(a)* gene. The *aph4* gene encodes for hygromycin B phosphotransferase which allows cells containing this gene to grow on medium containing hygromycin. Post-transformation, *Agrobacterium* were eliminated from tissues by culture on antibiotic-containing medium. This technique using disarmed *Agrobacterium* where the gene involved in pathogenicity are removed, followed by selection has a history of safe use and has been used for transformation of a variety of plant tissues for over 20 years (Howard, *et al.* 1990).

DNA sequences inserted into cotton Event COT102
Data supplied in the petition and reviewed by APHIS (petition pages 31-57) support the conclusion that Event COT102 contains the following sequences: 1) a modified actin (Act2) promoter from *Arabidopsis thaliana*, 2) the *vip3A(a)* gene from Bacillus thuringiensis strain AB88, 3) the 3'nontranslated terminating sequences of the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens*, 4) the ubiquitin 3 promoter (Ubq3int) from *Arabidopsis thaliana*, 5) the *aph4* gene from *Escherichia coli*. The *nos* sequence is from a soil-inhabiting bacterial plant pathogen, *Agrobacterium* sp. doesn't encode a protein; it does not cause plant disease and has a history of safe use in a number of genetically engineered plants (e.g., corn, cotton and soybean varieties).

Evaluation of intended effects

As a result of introduction of the *vip3A(a)* gene into the cotton genome, the resulting plants are resistant to certain lepidopteran pests (petition Table 1.1 and pages 64-73). The Event COT102 VIP3A protein is identical to that encoded by the native *Bacillus thuringiensis* protein (Estruch *et al.*, 1998; Warren *et al*., 1996) with the exception of a single amino acid difference at position 284. The native *vip3A(a)* gene encodes a lysine, while the Event COT102 *vip3A(a)* gene encodes a glutamine. Because the amino acid replacement has similar biochemical properties, the replacement does not constitute a biologically significant difference.

Analysis of inheritance
Data was provided and reviewed by APHIS that demonstrates stable integration and inheritance of the *vip3A(a)* and *aph4* genes and their associated regulatory sequences over several breeding generations. Statistical analyses show that insect resistance is inherited as a dominant trait in a typical Mendelian manner (petition pages 57-59).

Analysis of gene expression
Data on VIP3A and APH4 leaves in different plant tissue types was collected from field trials conducted at several locations. Using standard laboratory ELISA techniques VIP3A and APH4 protein concentrations were determined in cotton leaves, squares, roots, bolls, seeds, pollen, fiber and nectar. VIP3A protein was detected (petition Tables 6.1, 6.2, 6.5, 6.6, 6.7) in leaves, squares, roots, bolls, seeds, and pollen, but not in nectar. The maximum value detected (VIP3A per dry weight tissue) was 136 µg /g in leaves at the squaring stage, 10 µg/g in leaves pre-harvest, 24 µg /g in squares, µ7.8 g/g in roots, 3.7 µg/g in seeds and 1.1 µg/g in pollen. VIP3A was not detected in either cotton fiber or nectar. The APH4 protein was not consistently detected in tissues sampled (petition Tables 6.3, 6.4, 6.5, 6.6) including leaves, squares, roots, bolls, seeds, and nectar. When found APH4 could only be detected at extremely low levels and below the Lower Limit of Quantification. The Lower Limit of Quantification for APH4 was 150 ng APH4 / g dry weight. APH4 was detected in pollen at 2.3 µg /g.

By surveys of naturally occurring *Bacillus* sp. strains, *vip3A*-like genes are widely prevalent in nature (Estruch, *et al*., 1996; Guttmann & Ellar 2000; Rice 1999). *B. thuringiensis* strains have been used for decades in agriculture as the basis for microbial pesticides. Syngenta examined eight EPA registered formulations of Bt-based microbial insecticides by ELISA, and found all eight contained cross reactive VIP3A proteins of comparable molecular weight (petition page 160). Thus, *vip3A(a)* or *vip3A(a)*-like genes appear to commonly occur in *B. thuringiensis* strains and are present in EPA-registered microbial pesticides. *Bacillus thuringiensis* strains are bacteria commonly found in soils around the world, but are not plant pathogens. The VIP3A protein is devoid of inherent plant pest characteristics. The APH4 protein was introduced as a selectable marker to produce transformed plants. The *aph4 gene* (Kaster *et al*., 1983; Waldron, 1997) isolated from *E. coli* encodes the 341-amino acid enzyme, hygromycin B phosphotransferase (APH4). APH4 is devoid of inherent plant pest characteristics.

Analysis of the intended trait

To evaluate Event COT102 in different environments under standard growing conditions field trials were conducted in the U.S. (Appendix B of this EA) and in Argentina, China, Australia, South Africa, Costa Rica and Vietnam. Standard field trials evaluated 1) agronomic performance, 2) disease and pest resistance performance, 3) insect efficacy and 4) seed germination. Standard industry farming practices for the various locales was utilized in these trials. In general no significant differences were observed. When differences were observed these were not consistent across locations or from year to year, indicating these differences were due to a normal background of genetic variability as expressed in different environmental conditions. As intended, Event COT102 plants exhibited some resistance to the lepidopteran insect pests. However, consistent

control was not always demonstrated, because the target pest populations were sometimes too low to provide statistically significant data.

Analysis of possible unintended effects

To assess possible effects from introduction of the *vip3A(a)* and *aph4* gene and their associated regulatory sequences, both qualitative and quantitative data addressing disease susceptibility and overall agronomic performance were collected. APHIS reviewed data submitted by the petitioner describing these trials, conducted over several years in a variety of locations (petition pages 74 – 88). Most of these data were derived from Event COT102 plants grown in field tests in conditions similar to those found in typical cotton cultivation in the United States. Out of over 60 field trials conducted in the U.S. from 2000-2002, no significant differences were noted in disease susceptibility or non-target pest damage. However, non-target insects were generally found at higher population in Event COT102 and the parental line Coker 312 as compared with the chemical treated controls. Other phenotypic characterizations comparing Event COT102 with the parental control were also completed. Agronomic property, and disease and insect susceptibility data (see Appendix D for data summary) was provided by Syngenta and assessed by APHIS on general plant growth, germination, morphology, reproductive traits, and fiber quality. Qualitative and quantitative observations indicated a lack of biologically meaningful differences from control lines or differences outside the range of conventional cotton norms. Expression of VIP3A or APH4 in Event COT102 cotton is not expected to cause plant disease or influence susceptibility of Event COT102 or its progeny to diseases or other pests.

In addition to field studies on agronomic parameters, Syngenta analyzed Event COT102 for compositional changes as part of their submission to FDA in the consultation process. APHIS reviewed the data on protein, fat, ash, carbohydrate, fatty acids, moisture, key minerals, fiber content, and amino acids in seeds. All analyses fell within the range of values observed for the parental cotton cultivar, providing additional evidence that Event COT102 cotton does not exhibit unexpected or unintended effects.

Cotton plants produce the toxic compound gossypol and cyclopropenoid antinutrients. Syngenta analyzed the levels of the gossypol and cyclopropenoids in Event COT102 plants and APHIS found that they were indistinguishable from those measured in the parental cotton cultivar (petition page 96 & 97).

Event COT102 contains two additional protein products when compared with its untransformed parent Coker 312. The first protein VIP3A originating from *B. thuringiensis* is selectively active against certain lepidopteran insects. VIP3A is similar to Cry proteins which have a history of safe use, by its targeting receptors found in insect midgut (Lee, *et al*., 2003). However, because the VIP3A DNA sequence and the insect target receptor is different for the Cry proteins, its mode of action is unique. Thus, the VIP3A may provide novel methods for control of certain lepidopteran insects. The second protein APH4 originating from *E. coli* encodes for hygromycin B phosphotransferase was used as a selectable marker in the development of the Event COT102. Expression of the APH4 protein in plant cells allows for these cells to grow on media containing hygromycin. The APH4 protein detoxifies Hygromycin and a narrow range of structurally related microbial antibiotics such as Destomycin A and B, but not gentamycin, kanamycin, neomycin,

streptomycin, or tobramycin (Rao *et al*., 1983). The potential environmental effects data due to expression of these proteins was presented by Syngenta in the petition, reviewed by APHIS and is summarized in Appendix D of this Environmental Assessment.

Potential Environmental Impacts Based on the Relative Weediness of Cotton Event COT102 Compared to Currently Cultivated Cotton Varieties

APHIS evaluated whether Cotton Event COT102 would be any more likely to become a weed than its non-transgenic counterpart or than other cotton varieties currently offered for commercial use. The cultivated cotton from which these Cotton Events are derived, *Gossypium hirsutum*, is not typically considered a weed species in the United States or other countries (Reed, 1977; Muenscher, 1980; Holm *et al*., 1977, 1997, USDA NRCS, 2001) nor is it listed in the Weed Science Society's Composite List of Weeds (Weed Science Society of America, 1989). However, cotton has some characteristics as a weed, and the Southern Weed Science Society lists *G. hirsutum* as a potential weed in southern Florida (Southern Weed Science Society, 1998, http://plants.usda.gov/cgi_bin/invasive_one.cgi?pub=SWSS). Without human intervention, such as the typical agricultural practices, the cotton plant is a perennial, surviving many years if conditions allow. Cotton does not tolerate cold conditions, and only Hawaii, southern Florida, and Puerto Rico remain warm enough to allow cotton plants to survive the winter (Smith and Cothren, 1999).

APHIS evaluated data in the petition on the agronomic properties and pest susceptibility of Cotton Event COT102 to substantiate that these transgenic plants are similar in growth and development to the parental cotton line. Some minor statistically significant differences were noted between the means at field test locations for Coker 312 and Event COT102. When differences were detected they were not consistent across field test sites. These variations were slight and they would not be expected to increase weediness. Cotton Event COT102 plants have been grown in more than 60 field trials in the United States, since 2001. Quantitative and qualitative field observations of the plants indicate that Cotton Event COT102 plants are similar to their parental line. In addition to the results summarized above, APHIS notes that there have been no reports of increased weediness associated with other lepidopteran insect resistant cotton lines that have been granted nonregulated status. A comparison of environmental impacts of biotechnology derived and traditional cotton crops has not identified weediness associated with insect resistant cotton lines being grown in the U.S. (Carpenter *et al*., 2002).

Potential Environmental Impacts from Gene Introgression from Cotton Event COT102 to Sexually Compatible Relatives

Cotton Event COT102, like other cotton, can pass its traits to offspring by transmitting pollen to other plants which are sexually compatible; in this case, some species of the genus *Gossypium* (see Appendix A, http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm).

APHIS considered whether such crosses are likely to occur when Cotton Event COT102 is grown and whether the offspring from such crosses are more likely to pose any greater risk of weediness than crosses of other cotton cultivars with these sexually compatible species.

The genus *Gossypium* contains approximately 50 species, of which generally four species are cultivated for the cotton fibers that are attached to the seeds. Cotton Event COT102 is *Gossypium hirsutum*, the cotton species referred to as upland cotton. Most of the cotton grown in the United States is *G. hirsutum*, but Pima cotton (*G. barbadense* L.) is also grown. In addition to these cultivated species, there are two wild *Gossypium* species in the United States, *G. thurberi* and *G. tomentosum*, which are found in the mountains of southern Arizona and in Hawaii, respectively. Neither *G. thurberi* nor *G. tomentosum* are listed as weeds, either on the Federal nor State lists of noxious weeds (http://plants.usda.gov/cgi_bin/noxious.cgi?earl=noxious.cgi). An older literature citation lists *G. tomentosum* as a weed of unknown importance in its range (Holm *et al*.,1979).

Genetic incompatibility precludes successful crosses of *G. hirsutum* with *G. thurberi*, but the compatibility of crosses between *G. hirsutum* and *G. tomentosum* is less understood. Some researchers have speculated that crosses may have occurred in the evolution of *G. tomentosum* but genetic exchange appears to be rare. Part of the rarity may be due to the fact that *G. hirsutum* is largely self-pollinating rather than cross-pollinating. In addition, the pollinators of *G. hirsutum* tend to be bumblebees whereas moths pollinate *G. tomentosum*. Also, *G. hirsutum* flowers are sexually receptive for pollination during the day while *G. tomentosum* receptivity is at night. APHIS has consulted with Dr. Derral Herbst, a prominent botanist in Hawaii with decades of experience and an author of the definitive "Manual of the Flowering Plants of Hawaii" recently revised in 1999 (personal communication with Bruce MacBryde, Ph.D., APHIS, Jan. 30, 2004). Dr. Herbst, indicated that based on his field work over the years and on herbarium collections at the Bishop Museum, which houses the Hawaiian Biological Survey, he has not seen a hybrid between *G. tomentosum* and either of the cotton species which have naturalized there, *G. hirsutum* and *G. barbadense*. He was also of the understanding that genetic barriers between the species result in weak, sterile F2 generations.

Even in cases of complete genetic compatibility (*G. hirsutum* crossed with another *G. hirsutum*), successful outcrossing is severely limited when the plants are separated by more than 660 feet. In experiments designed to detect gene flow in Mississippi, detectable gene flow was very low (less than 1%) when *G. hirsutum* plants were 25 meters apart (Umbeck, 1991). Cotton breeders and seed producers routinely use field data to decide on the isolation distances for the production of certified and foundation cotton seeds (660 and 1320 feet, respectively).

Because it is unlikely that *G. hirsutum* will readily cross with *G. thurberi* and *G. tomentosum*, it is unlikely that the either the VIP3A or the APH4 protein will introgress from Cotton Event COT102 into *G. thurberi* and *G. tomentosum*. In the registration requirements for other Bt cotton varieties (Bollgard and Bollgard II), the EPA stipulated geographic restrictions to mitigate gene flow to sexually compatible relatives in parts of the United States where *G. thurberi* and *G. tomentosum* are found, imposing conditions based on reproductive compatibility in crosses of *G. hirsutum* to other *G. hirsutum* (US EPA, 2001a and 2002). As summarized above, however, such crosses between the cultivated and wild cottons do not appear to occur in nature. There are no reports of intermediate cotton types that one would expect in the areas where *G. hirsutum* has been grown in proximity to *G. thurberi* and *G. tomentosum*.

Outcrossing considerations may be different in other parts of the world. For example, other species which might potentially intercross with *G. hirsutum* cultivars include *G. mustelinum* in northeastern

Brazil and *G. lanceolatum* in mid-Mexico (Fryxell 1979). Other Old World *Gossypium* sp. cottons are diploid as are the other five genera of cotton relatives among the Gossypieae Tribe (Fryxell, 1979). The likelihood of successful intercrossing with these diploid species may be quite low because of the production of triploids that are likely to be sterile. This is consistent with the fact that such intergeneric crosses have not been observed (Fryxell, 1979).

On July 2001, EPA published its final FIFRA regulations regarding plant incorporated protectants, of which the Bt Cry proteins are an example (http://www.epa.gov/pesticides/biopesticides/pips/pip_rule.pdf). In a statement in its final rule on plant-incorporated protectants, EPA found that "weediness is generally thought to be due to a multiplicity of factors" (US EPA, 2001b). The National Research Council has also concluded that "genetically modified crops are not known to have become weedy through the addition of traits such as herbicide and pest resistance" (National Research Council, 1989).

Potential Environmental Impacts on Non-Target Organisms, Including Beneficial Organisms and Threatened and Endangered Species

APHIS evaluated the potential for Event COT102 plants and their products to have damaging or toxic effects directly or indirectly on non-target organisms. Non-target organisms considered were those representatives of the exposed agricultural environment, including those that are recognized as beneficial to agriculture or as threatened or endangered in the United States. APHIS also considered potential impacts on other "non-target" pests since such impacts could potentially change agricultural practices.

Potential environmental impacts on non-target, non-lepidopteran pests
Target pests of the VIP3A protein expressed in cotton Event COT102 include certain lepidopteran larvae. Although laboratory studies have shown many lepidopteran pests are sensitive to the VIP3A protein (petition Table 1.1 and letter from Syngenta dated April 20, 2004), other lepidopteran pests are insensitive (petition Table 1.2 and letter from Syngenta dated April 20, 2004). Event COT102 cotton is intended to control the cotton bollworm (*Helicoverpa zea*), tobacco budworm (*Heliothis virescens*), pink bollworm (*Pectinophora gossypiella*), fall armyworm (*Spodoptera frugiperda*), beet armyworm (*Spodoptera exigua*), soybean looper (*Pseudoplusia includens*) and cabbage looper (*Trichoplusia ni*). The lack of sensitivity to the VIP3A protein of non-target lepidopteran and non-lepidopteran invertebrate species, including representatives from the Coleoptera, Diptera, Homoptera and Thysanoptera orders, has been verified in laboratory studies (petition, Table 1.3, environmental assessment Appendix D).

Potential environmental impacts on non-target organisms, including beneficial organisms
APHIS evaluated the results of several studies submitted that were designed to evaluate the sensitivity of representative non-target organisms to VIP3A protein. Test substrates included corn plant material (e.g., corn grain, leaf or pollen) expressing modified VIP3A protein or VIP3A purified from an *E. coli* bacterial strain engineered to express the protein. Syngenta verified that the bacterially-produced VIP3A, as purified and prepared for these studies, was similar enough in its biochemical properties (molecular weight, amino acid sequence, and lack of glycosylation) and in its biological activity against lepidopteran larvae, to warrant its use as a test substance comparable to VIP3A as produced in Event COT102 plants (Appendix D). When conducting laboratory tests,

foods used by test species in their relevant habitat are preferred (EPA SAP, 2002; EPA SAP, 2004). However, the use of corn-derived plant material rather than cotton-derived plant material is acceptable because cotton-derived plant material contains gossypol and other possible plant toxicants that may adversely affect non-target organisms.

Acute dietary toxicity studies were conducted in laboratory tests by feeding VIP3A corn pollen to beneficial arthropods including honey bee (*Apis mellifera*) adults and larvae, adult lady beetles (*Coleomegilla maculate*), adult green lacewings (*Chrysoperla carnea*) and the freshwater invertebrate *Daphnia magna*. The level of VIP3A expression was higher in the corn pollen used than in Event COT102 cotton pollen (see petition, Table 7.2). Representative decomposers were evaluated using VIP3A expressing corn tissue including a 28 day chronic effects study on survival and reproduction of the soil-dwelling arthropod Collembola (springtails) (*Folsomia candida*) and an acute toxicity study with earthworms. Expression levels of VIP3A are greater in young cotton leaves than young corn leaves (see petition, Table 7.2). Therefore, laboratory studies conducted with lyophilized corn leaf tissue (e.g., Collembola and earthworms) were provided with enough test material to account for the lower expression of VIP3A in corn than in cotton tissue. Northern bobwhite quail, catfish and mice toxicity tests were conducted with corn grain containing the VIP3A protein. VIP3A expression levels are greater in corn seeds and grain than cotton (petition, Table 7.2). All of the organisms evaluated in the Tier 1 dietary toxicity studies were exposed to higher levels of the VIP3A protein (six to 2100 fold levels) than they would be exposed to in the field (petition Table 7.1).

Results of these studies indicate that no deleterious effects on non-target organisms would be expected due to incidental exposure or feeding on Cotton Event COT102 (petition Table 7.1). This analysis took into consideration the levels of the VIP3A protein in different tissues of Event COT102 (petition Table 7.3), the environmental fate and likely routes and levels of exposure to plant tissue or residues of this tissue that contain the active toxin, and dietary preferences. Since predatory insects will have limited direct exposure to VIP3A insecticidal proteins expressed in Event COT102, little impact is expected for these species other than a possible shift to non-lepidopteran prey since lepidopteran populations in these cotton events are expected to be reduced. However, the expectation that beneficial insects will not be adversely affected must be verified. Therefore, three beneficial predators or parasitoids are typically evaluated for plant incorporated protectants. Since only two predators, lady beetles and green lacewings, were tested for VIP3A and no parasitic Hymenoptera were evaluated in the laboratory, EPA will be requiring an additional non-target insect study (e.g., the minute pirate bug) as a condition of registration (personal communication, Leonard Cole, EPA on October 21, 2004).

A large-scale field study was summarized in pages 146-150 of the petition. This two year field study evaluated the abundance and diversity of invertebrates from visual plant inspections, litter, pitfall and sticky trap sampling in a corn hybrid containing both VIP3A and Cry1Ab proteins. Using hybrid corn containing both Bt proteins was designed to give a conservative estimate of effects on invertebrate populations. During the two years of documenting invertebrate populations at the ground, aerial and plant level, over 200,000 organisms representing 78 families were recorded. Results of these studies indicate no adverse effects to non-target organism including predators, parasitoids (e.g., parasitic Hymenoptera), decomposers and herbivores exposed to or feeding on corn expressing the VIP3A protein. Although this field study considers VIP3A and Cry1Ab proteins

expressed in corn hybrids, this data may be considered for cotton since many of the beneficial organisms evaluated such as predatory lady beetles and minute pirate bugs occur in both corn and cotton fields. Additional field studies conducted with Cotton Event COT102 will be required on a commercial-scale by the EPA as a condition of registration (personal communication, Leonard Cole, EPA, October 21, 2004). Unintended effects are not expected based on the known host specificity (petition Table 1.1 for a list of sensitive pests, Table 1.2 and 1.3 for a list of insensitive insects). In addition, APHIS realizes that EPA will conduct a non-target risk assessment prior to approving a commercial registration of Event COT102 cotton. All non-target organism studies submitted to the EPA will be shared with APHIS.

Selectivity and environmental impacts of the insecticidal proteins to Lepidoptera

The VIP3A insecticidal control proteins are active against a certain lepidopteran larvae (petition Table 1.1) including the black cutworm (*Agrotis ipsilon*), fall armyworm (*Spodoptera frugiperda*), beet armyworm (*Spodoptera exigua*), tobacco budworm (*Heliothis virescens*) and corn earworm (*Helicoverpa zea*) (Estruch *et al*. 1996, Yu 1997). In some instances, VIP3A has 260x the activity against black cutworm than Cry1A proteins (Estruch *et al.* 1996). VIP3A is not active against some lepidopterans (petition Table1.2); for instance the European corn borer (*Ostrinia nubilalis*) and monarch butterfly (*Danaus plexippus*) are not susceptible to VIP3A expressed in Event COT102 cotton (Yu, *et al*. 1997; Lee, *et al.* 2003).

VIP3A proteins have been isolated from *Bacillus thuringiensis* strain AB88 and are expressed during the vegetative and sporulation developmental stages, whereas Bt delta-endotoxins are only expressed during the sporulation phase (Estruch, *et al*. 1996). In general, ingestion of VIP3A proteins by susceptible insects leads to similar symptoms as ingestion of Bt delta-endotoxins such as feeding cessation, loss of gut peristalsis, insect paralysis and death. However, a study by Lee, *et al*. (2003) found that VIP3A "utilizes a different molecular target and forms distinct ion channels compared to Cry1Ab." Ingestion of VIP3A by susceptible insects has been shown to lead to gut paralysis at concentrations 4 ng/cm^2. A concentration of 40 ng/cm^2 has caused lysis of gut epithelium cells and death of susceptible larvae (Yu, *et al*. 1997).

The VIP3A specificity of insecticidal activity is dependent upon binding to specific receptors present in the mid-gut of lepidopteran insects different than the Cry protein receptors (Lee, *et al.* 2003). These insecticidal proteins are not expected to adversely affect other invertebrates or vertebrate organisms, including non-target birds, mammals and humans (Appendix D), because these organisms would not be expected to contain the receptor protein found in the insect's midgut. APHIS evaluated laboratory and field studies on representative species that support these expectations. In conclusion, Event COT102 contains *vip3A(a)* that produce no unintended effects, are stably inherited in Mendelian fashion.

Potential environmental impact due to the selectable marker

The selectable marker *aph4* gene encodes for hygromycin resistance. Hygromycin resistance genes are found in *E. coli* and *Streptomyces hygroscopicus*, both of which are widespread in the environment. Hygromycin B phosphotransferase may be used to as an antihelmintic (de-worming agent) in swine and poultry (http://www.efsa.eu.int/science/gmo/gmo_opinions/384_en.html). To evaluate safety, the European Food Safety Authority reviewed the antibiotic selection markers used

in genetically engineered plants (http://www.efsa.eu.int/science/gmo/gmo_opinions/384_en.html). Various antibiotic resistance genes were assigned into groups based on the criteria of therapeutic use in humans and in animals and presence in the environment; Group I is composed of kanamycin and hygromycin resistance. The opinion states that because of the frequency of horizontal transfer from plants to other organisms is very rare, previous existence in the environment and the history of safe use of the kanamycin resistance, that there is no rationale for restricting group I antibiotics.

The APH4 protein was not detected consistently in any Event COT102 tissues sampled (petition Tables 6.3, 6.4, 6.5, 6.6), except for pollen. When found in tissues other than pollen, the APH4 protein could only be detected at extremely low levels and below the Lower Limit of Quantification. The Lower Limit of Quantification for APH4 was 150 ng APH4 / g dry weight. The EPA has granted an exemption from the requirement of tolerance for APH4 (http://www.epa.gov/fedrgstr/EPA-PEST/2004/April/Day-07/p7866.htm) which eliminates the need to establish a maximum permissible level for residues of hygromycin B phosphotransferase (APH4) when used as an inert ingredient in plant-incorporated protectants. The expression of the APH4 protein in cotton plants is not expected to have deleterious effects or significant impacts on non-target organisms, including beneficial organisms, based on data provided in the petition and the general knowledge obtained from a history of use of antibiotic resistant genes. In conclusion, Event COT102 contains *aph4* that produces no unintended effect and is stably inherited in Mendelian fashion.

When an enzyme is present in a new background, as APH4 in Event COT102 there is concern that novel and potentially toxic products may be produced. Because APH4 acts only on a narrow range of microbial antibiotics that are structurally related to hygromycin (Rao *et al*., 1983) and similar substrates are not known to be present in plants (Syngenta correspondence November 4, 2004), novel toxic products are highly unlikely to be produced in Event COT102. In addition, even though hygromycin is a naturally produced microbial antibiotic, it is expected to be produced by microbes in the environment in extremely small quantities. Therefore, because it is extremely unlikely that plants would be exposed to hygromycin in the environment, novel compounds are very unlikely to be produced. In conclusion the APH4 or the products of its enzymatic reactions are not expected present any new plant pest risk and will not harm threatened and endangered species.

Over the course of evolutionary time, horizontal transfer between plants may occur (Palmer, *et al*., 2000). This very rare transfer would not present a new plant pest risk because APH4 and VIP3A do not confer any plant pest properties. Thus, because *aph4* and *vip3A(a)* genes are already present in the environment and do not confer any plant pest properties and the rate of horizontal transfer is very rare, Event COT102 does not present any new plant pest issues.

Potential impact on threatened and endangered species

APHIS also considered the potential impact that a nonregulated status of Cotton Event COT102 might have on organisms which are on the Federal List of Threatened and Endangered Species. The incorporation of VIP3A into cotton production may further reduce chemical pesticide use and the concomitant potential for negative impact to non-target species via chemical spray drift, bioaccumulation in food chains, and the contamination of surface and groundwater sources. APHIS did not focus its analysis extensively on such potential benefits, but examined the potential harm that

might result to threatened and endangered species which are similar to the target insect pests and therefore likely to be sensitive to VIP3A if ingested. The threatened and endangered species most likely to be negatively affected by these proteins would be lepidopteran insects. Since it is not possible to use such species to quantify sensitivity to VIP3A, the APHIS evaluation started with the assumption of some toxicity and focused instead on whether it is likely that these species would be exposed to the toxins expressed in the subject transgenic cotton lines. Exposure of these species is only likely if the species occur in the areas where cotton is grown, because cotton plant parts (seeds, pollen, crop debris) are not readily transported long distances without the intervention of humans.

The APHIS environmental assessment for the petition (00-342-01p) for deregulation of a Bt cotton, Bollgard II, which expresses both Cry1Ac and Cry2Ab, examined the potential impacts on threatened and endangered species as did EPA's Biopesticides Registration Action Document for this product (http://www.epa.gov/pesticides/biopesticides/ingredients/tech_docs/brad_006487.pdf). No listed species have been identified that would be expected to be impacted. In the states which grow cotton, only California, Florida, and North Carolina have lepidopteran species that are on the Federal endangered species list. These species do not feed on cotton and their habitats do not overlap with cotton fields. Additionally, cotton pollen is heavy and is not expected to drift into these habitats in sufficient quantities onto host plants of the larvae forms of these species to have an effect.

Of the 15 threatened or endangered California species, 13 are found in habitats which are far from the cotton growing areas in the Central Valley of California. Only one species, the Quino Checkerspot (*Euphydryas editha quino*), has populations in a cotton producing county. This nymphalid butterfly is found in both upland sage scrub or chaparral communities and in meadows (Fish and Wildlife Service, 2001). Its host plants, the dotseed plantain and the exerted Indian paintbrush are both adequate hosts for the larvae only in late winter and spring, as the vegetation mostly dies back in the summer. The adults emerge in early or midspring and lay eggs which continue to grow until the summer dries the vegetation. A larval diapause stage occurs until the late winter, when host plants again flourish and pupation occurs. It is unlikely that significant insect presence would overlap with cotton cultivation, although in some years this might occur. Meadows in the vicinity of cotton and other agricultural production are likely to have been used for growing crops over many years, and that is one reason why this insect has become endangered. Thus, geographic isolation is likely to prevent Event COT102 plants from impacting this butterfly. The Fish and Wildlife Service has not described any agricultural impact on the populations of the Quino Checkerspot butterfly except the impact of livestock trampling the insect's host plants (Fish & Wildlife Service, 1997).

A second endangered lepidopteran species in California, the Kern Primrose Sphinx (*Euproserpinus euterpe*), may occupy habitat near cotton cultivation sites in Kern County, but this moth has not been detected since 1982. It was formerly collected within southern Kern County on a single ranch (see EPA assessment of threatened and endangered species for Bollgard II cotton http://www.epa.gov/pesticides/biopesticides/ingredients/tech_docs/brad_006487.pdf). Its host plant is evening primrose, *Camissonia* spp., which are distributed throughout Southern California and beyond.

In North Carolina, another endangered butterfly, the St. Francis Satyr (*Neonympha mitchellii francisci*) is known, although cotton cultivation near its known habitat is unlikely. This butterfly

lives in the boggy areas and wide wet meadows of the Ft. Bragg military base (Fish & Wildlife Service, 1994), an area where cotton cultivation is unlikely.

In Florida, the Schaus swallowtail (*Heraclides aristodemus ponceanus*) is a subtropical species which lives in the far southern portion of the state. It is most commonly found in Elliot Key and North Key Largo. Cotton is not cultivated in this region so exposure is very unlikely.

APHIS also considered threatened and endangered species other than lepidopterans. The petitions provided data which support the conclusion that the Bt proteins expressed in VIP3A cotton are not toxic to invertebrates other than lepidopterans. Data also corroborated that they are relatively non-toxic to vertebrates (e.g. fish, birds, and mammals). In total, these analyses, and the data submitted by Syngenta and information in the scientific literature indicate that Cotton Event COT102 will not cause harm to any threatened or endangered species.

Potential Impacts on Biodiversity

After careful evaluation, APHIS believes that Cotton Event COT102 exhibit no traits that would cause increased weediness, that cultivation of this Cotton Event should not lead to increased weediness of other cultivated cotton or other sexually compatible relatives, and is unlikely to harm non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service. Based on this analysis, APHIS believes that it is unlikely that Event COT102 or its progeny will pose a significant impact on biodiversity.

Potential Impacts on Agricultural and Cultivation Practices

APHIS considered the potential impacts of Cotton Event COT102 on current agricultural practices in the United States. The potential impacts on organic farming and on minorities and children were also considered. APHIS also considered any potential cumulative effects that might arise from the use of Event COT102 or its progeny in agricultural production.

Impacts on current agricultural practices

The Economic Research Service of the USDA reports that in the year 2000 an estimated 35% of cotton acreage in the United States was planted with approved genetically engineered varieties containing the Cry1Ac protein to deter feeding damage from lepidopteran insect pests (http://www.ers.usda.gov/publications/aer810/aer810.pdf). The comparative environmental impacts and impacts on agricultural practices from biotechnology-derived and traditional crops, including cotton, were summarized in a report by Carpenter *et al*., 2001, published by the Council for Agricultural Science and Technology. Event COT102 was developed to provide another option for control of these pests and prolong the useful lifetime of Cry-protected varieties already in commercial use. The possible commercial use of varieties based upon Event COT102 may enable a continued reduction in the use of insecticides to control lepidopteran pests of cotton. The Economic Research Service of the USDA has reported a reduction in pesticide use by cotton growers using the first generation of Cry-protected cotton varieties (http://www.ers.usda.gov/publications/aer810/aer810.pdf). Growers have still had to use chemical and other strategies to control cotton pests that are not affected by the Cry protein. However, it is believed by both growers and researchers that reduced reliance on chemical pesticides in cotton

cultivation allows populations of beneficial organisms (insects, mites, wasps, etc) to increase to levels that can exert effective control of some of the cotton pests.

Bt-derived insecticides are of importance because of high selectivity against certain lepidopteran pest. Event COT102 differs from currently registered Bt-cotton varieties because it operates through a novel mechanism of action (Lee, *et al.*, 2003) in that VIP3A proteins target a different insect gut receptor compared with Cry-protein receptor. VIP3A high selectivity against certain lepidopteran pests and novel mode of action indicates that adoption of Event COT102 and progeny may reduce the potential of build up of Bt-resistance in populations of target insects. The adoption of Bt-cotton in the U.S. has reduced pesticide application (http://www.ers.usda.gov/publications/aer810/) and the VIP3A derived products may provide cotton growers an additional insect control tool in the management of pest resistance.

Potential impacts on organic farming

The National Organic Program (NOP) administered by USDA's Agricultural Marketing Service (AMS) requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances from adjoining land that is not under organic management. Organic production operations must also develop and maintain an organic production system plan approved by their accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. Excluded methods include a variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes.

Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. This oversight includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods.

The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards. The unintentional presence of the products of excluded methods will not affect the status of an organic product or operation when the operation has not used excluded methods and has taken reasonable steps to avoid contact with the products of excluded methods as detailed in their approved organic system plan. Organic certification of a production or handling operation is a process claim, not a product claim.

Several transgenic cotton varieties resistant to lepidopteran insects are already in widespread use by growers. Varieties derived from cotton Event COT102 should not present new and different issues than those with respect to impacts on organic farmers. APHIS has considered that it is possible that the genes from this Event COT102 could move to cotton in an adjacent field via cross-pollination. All cotton, whether genetically engineered or not, can transmit pollen to nearby fields, and a very small influx of pollen originating from a given cotton variety does not appreciably change the characteristics of cotton in adjacent fields. As described previously in this assessment, the rate of cross-pollination from one field to another is expected to be quite low, even if flowering times coincide. The frequency of such an occurrence decreases with increasing distance from the pollen

source such that it is sufficiently low at 1320 feet away to be considered adequate for production of even the most restrictive standard for foundation cotton seeds (see footnote 19 for the table found at http://www.aphis.usda.gov/brs/isolate.html). Organic cotton growers could use isolation distance or differences in planting time to minimize the potential for any unwanted outcrossing of transgenic cotton to their crop.

It is not likely that organic farmers, or other farmers who choose not to plant transgenic varieties or sell transgenic grain, will be significantly impacted by the expected commercial use of products derived from cotton Event COT102 since: (a) nontransgenic cotton will likely still be sold and will be readily available to those who wish to plant it; (b) farmers purchasing seed will know this product is transgenic because it will be marketed and labeled as VIP3A lepidopteran resistant, and based on the IRM plan farmers will be educated about recommended management practices.

Potential impacts on minorities, low income populations and children

Potential impacts on humans, including minorities, low income populations, and children was also considered. In accordance with the directive specified in Executive Order 13045, APHIS has attempted to identify and assess environmental health or safety risks that might disproportionately affect children. APHIS also considered any possible adverse impacts on minorities and low-income populations as specified under Executive Order 12898 published February 11, 1994. Collectively, the available mammalian toxicity data and history of safe use of microbial Bt products and other cotton varieties expressing Cry proteins, and mammalian toxicity data of cotton expressing VIP3A supports the safety of cotton Event COT102 and their products to humans, including minorities, low income populations, and children who might be exposed to them through agricultural production and/or processing. No additional safety precautions would need to be taken in consideration of these groups. None of the impacts on agricultural practices described above are expected to have a disproportionate adverse effect on minorities, low-income populations, or children, and may in fact provide benefits. As noted above, if approved for cultivation, the cotton derived from Event COT102 is expected to further decrease reliance on chemical insecticides used to control lepidopteran pests, some of which are less favorable with respect to environmental and human toxicity.

Potential impacts on raw or processed agricultural commodities

Our analysis of data on agronomic performance, disease and insect susceptibility, and compositional profiles of the seeds and fiber indicate that cotton Event COT102 is similar to its non-transgenic parent counterpart and other cultivars of *G. hirsutum*. APHIS does not foresee either a direct or indirect plant pest effect on any raw or processed plant commodity.

Potential environmental impacts outside the United States

APHIS has also considered potential environmental impacts outside the United States and its territories associated with a determination of nonregulated status for cotton Event COT102. It should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new cotton cultivars internationally, apply equally to those covered by an APHIS determination of nonregulated status under 7 CFR Part 340. Any international traffic in cotton subsequent to these determinations would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC). The IPPC has set a

standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (116 countries as of June, 2001). In addition, issues that may relate to commercialization and transboundary movement of particular agricultural commodities produced through biotechnology are being addressed in international forums and through national regulations. The Cartegena Protocol on Biosafety is a treaty under the Convention on Biological Diversity (CBD) that established a framework for the safe transboundary movement, with respect to the environment and biodiversity, of living modified organisms (LMOs), including those developed through biotechnology. The protocol came into force on September 11, 2003 and 82 countries are parties to it as of Jan. 21, 2004 (see http://www.biodiv.org/biosafety/default.aspx). Although the United States is not a party to the CBD, and thus not a party to the Cartegena Protocol on Biosafety, US exporters will still need to comply with domestic regulations of importing countries that are parties to the Protocol have put in place to comply with their obligations. The first intentional transboundary movement of LMOs will require consent from the importing country under an advanced informed agreement (AIA) provision and the required documentation. To facilitate compliance with obligations to this protocol, the US Government has developed a website that provides the status of all regulatory reviews completed for different uses of the product (http://usbiotechreg.nbii.gov/database_pub.asp). This data is available to the Biosafety Clearinghouse database that contains regulatory decisions for LMOs that may be subject to the Biosafety Protocol.

APHIS continues to play a role in working toward harmonization of biosafety and biotechnology guidelines and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States. NAPPO's Biotechnology Panel advises NAPPO on biotechnology issues as they relate to plant protection and NAPPO has developed a standard for the Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries (see http://www.nappo.org/Standards/Std-e.html). APHIS also participates regularly in biotechnology policy discussions at forums sponsored by the European Union and the Organization for Economic Cooperation and Development. APHIS periodically holds discussions on biotechnology regulatory issues with other countries (e.g. with Canada, Mexico, Argentina, Brazil, Japan, China, Korea to name a few) and has participated in numerous conferences intended to enhance international cooperation on safety in biotechnology. APHIS has sponsored several workshops on safeguards for planned introductions of transgenic crops most of which have included consideration of international biosafety issues. Mexico and Brazil, both of which have relatives of cotton that can potentially interbreed with it, have procedures in place that require a full evaluation of transgenic plants before they can be introduced into the environment and both countries have ratified the Cartegena Protocol. Many countries, including Australia, Mexico, South Africa, China, and Argentina are already growing other approved varieties of Bt cotton (Carpenter *et al*., 2002). APHIS does not expect a significant environmental impact outside the United States should nonregulated status be granted for the subject Cotton Event COT102.

C. Alternative C: Approval of the Petition in Part

EPA is currently reviewing the application to register cotton Event COT102 under its regulations for plant-incorporated protectants. EPA has the authority to impose geographic limitations on the use of specific pesticides and routinely does so to protect threatened and endangered species, as well as other non-target organisms. EPA and APHIS agree that the threatened and endangered lepidopteran

species do not typically feed on cotton so they are not likely to be exposed to the VIP3A protein. Cotton plants are not considered to be wind pollinated so it is not likely that the relatively heavy pollen grains will move from the cotton plants to rest on the surface of other substrates that will be ingested by these threatened and endangered lepidopteran species. All of the environmental considerations under Part B would be applicable to such a determination.

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VII. CONSULTATIONS

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Appendix A: Biology of cotton and potential for introgression into related species.

Cotton as a Crop

Four species of the genus *Gossypium* are known as cotton, which is grown primarily for the seed hairs that are made into textiles. Cotton is predominant as a textile fiber because the mature dry hairs twist in such a way that fine, strong threads can be spun from them. Other products, such as cottonseed oil, cake, and cotton linters are byproducts of fiber production.

Cotton, a perennial plant cultivated as an annual, is grown in the United States mostly in areas from Virginia southward and westward to California, in an area often referred to as the Cotton Belt (McGregor, 1976).

Taxonomy of Cotton

The genus *Gossypium*, a member of the Malvaceae family, consists of some 50 species, four of which are generally cultivated (Fryxell, 1984; Fryxell, 1992). The most commonly cultivated species, *G. hirsutum* L., is the subject of this Environmental Assessement. Other cultivated species are *G. arboreum* L., *G. barbadense* L., and *G. herbaceum* L.

Four species of Gossypium occur in the United States (Fryxell, 1979; Kartesz & Kartesz, 1980). *Gossypium hirsutum* is the primary cultivated cotton. *Gossypium barbadense* is also cultivated. The other two species, *G. thurberi* Todaro and *G. tomentosum* Nuttall ex Seemann, are wild plants of Arizona and Hawaii, respectively. *Gossypium tomentosum* is known from a few strand locations very close to the ocean.

Genetics of Cotton

At least eight genome designations, A, B, C, D, E, F, G and K, are found in the genus (Endrizzi *et al.*, 1985). Diploid species (2n=26) are found on all continents, and a few are of some agricultural importance. The A genome is restricted in diploids to two species (*G. arboreum*, and *G. herbaceum*) of the Old World. The D genome is restricted in diploids to some species of the New World, such as *G. thurberi*.

By far, the most important agricultural cottons are *G. hirsutum* and *G. barbadense*. These are both allotetraploids of New World origin, and presumably of ancient cross between Old World A genomes and New World D genomes. How and when the original crosses occurred have been subject to much speculation. Euploids of these plants have 52 somatic chromosomes, and are frequently designated as AADD. Four additional New World allotetraploids occur in the genus, including *G. tomentosum*, the native of Hawaii. *Gossypium tomentosum* has been crossed with *G. hirsutum* in breeding programs.

The New World allotetraploids are peculiar in the genus, because the species, at least in their wild forms, grow near the ocean, as invaders in the constantly disturbed habitats of strand and associated environs. It is from these "weedy" or invader species that the cultivated cottons developed (Fryxell, 1979).

Weediness of Cotton

Although the New World allotetraploids show some tendencies to "weediness" (Fryxell, 1979), the genus shows no particular weedy aggressive tendencies.

Pollination of Cotton

Gossypium hirsutum is generally self-pollinating, but in the presence of suitable insect pollinators can exhibit cross pollination. Bumble bees (*Bombus* spp.), Melissodes bees, and honey bees (*Apis mellifera*) are the primary pollinators (McGregor, 1976). Concentration of suitable pollinators varies from location to location and by season, and is considerably suppressed by insecticide use. If suitable bee pollinators are present, distribution of pollen decreases considerably with increasing distance. McGregor (1976) reported results from an experiment in which a cotton field was surrounded by a large number of honey bee colonies, and movement of pollen was traced by means of fluorescent particles. At 150 to 200 feet, 1.6 percent of the flowers showed the presence of the particles. The isolation distance for Foundation, Registered, and Certified seed in 7 CFR Part 201 is 1320 feet, 1320 feet, and 660 feet, respectively.

Research in Mississippi shows that pollen movement decreases rapidly after 40 feet (12 meters). Umbeck *et al.* (1991) studied pollen and successful gene movement of cotton in Mississippi test plots. Around a central transgenic test plot of 98,800 plants with rows running north-south, they planted 23 one-meter border rows of non-transgenic cotton to the east and to the west, and 25 meters of non-transgenic cotton border rows to the north and to the south, each divided into two 12.5 meter long plots. The border rows to the north and south were continuous with the transgenic rows. They took 32,187 seed samples from all border rows at bottom, middle, and top plant position (representing seasonal variation) and used a kanamycin resistance marker gene to test for seeds resulting from pollen movement out of the central transgenic plot. To the east and west, gene movement at the first row was 0.057 and 0.050, and dropped rapidly to row 8, and was not detected in subsequent rows to the east, and detected occasionally at <0.01 in rows to the west. Combined data for east and west border rows beyond row 9 gave total outcrossing of 0.0012. To the north and south, detections were totaled for each 12.5 meter block and gave figures of 0.0053 and 0.0047 for north and south inner block and 0.0015 and 0.0021 for north and south outer block.

Modes of Gene Escape in Cotton

Genetic material of *G. hirsutum* may escape from an area of cultivation by vegetative material, by seed, or by pollen. Propagation by vegetative material is not a common method of reproduction of cotton. Movement of seed can occur on farm implements such as planters and harvesters and can be minimized by cleaning of equipment between plots when separation of crop varieties is desired.

Movement of genetic material by pollen is possible only to those plants with the proper chromosomal type, in this instance only to those allotetraploids with AADD genomes. In the United States, this would only include *G. hirsutum*, *G. barbadense*, and *G. tomentosum*. *Gossypium thurberi*, the native diploid from Arizona with a DD genome, is not a suitable recipient. Movement to *G. hirsutum* and *G. barbadense* is possible if suitable insect pollinators are present, and if there is a short distance from transgenic plants to recipient plants. Physical barriers, intermediate pollinator-attractive plants, and other temporal or biological impediments would reduce the potential for pollen movement.

Movement of genetic material to *G. tomentosum* is less understood. The plants are chromosomally compatible with *G. hirsutum*, but there is some doubt as to the possibility for pollination. The stigma in *G. tomentosum* is elongated, and the plant seems incapable of self-pollination until acted upon by an insect pollinator, but flowers of *G. tomentosum* seem to be pollinated by moths, not bees. And they are receptive at night, not in the day. Most *Gossypium* flowers are ephemeral: they open in the morning and wither at the end of the same day. Both these factors would seem to minimize the possibility of cross-pollination. However, Fryxell (1979) reports that *G. tomentosum* may be losing its genetic identity from introgression hybridization of cultivated cottons by unknown means.

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Appendix B. List of APHIS authorizations to field tests of Syngenta Event COT102.

Notification number	States
00-122-04n	TX
00-301-03n	HI, TX
01-082-05n	AZ, GA, MS, SC, TX
01-078-20n	AZ, GA, MS, SC, TX
01-131-04n	CA
02-063-06n	CA, MS, TX
02-063-08n	AZ, GA, MS, SC, TX
02-063-09n	AZ
02-063-10n	AZ, GA, MS, TN, TX
02-072-15n	AL, GA, LA, MS, TX
02-072-17n	AL, GA, NC, LA, TX
02-086-15n	MS
02-086-14n	AR, CA, FL, MS, NC
02-093-02n	AZ
02-100-01n	AR
02-105-08n	CA
02-108-04n	AZ
02-133-04n	TX

Appendix C. Table comparing environmental fate and effects of Vip3A expressed in Syngenta Event COT102 with other insecticides used to control lepidopteran pests of cotton in the United States.

	VIP3A [1] [Bt protein]	Cry1F [2] [Bt protein]	Chlorpyrifos[3] (Lorsban) [organophosphate]	Permethrin[4] (Ambush/Pounce) [pyrethroid]
Environmental Fate	Soil degradation tests were conducted to determine if VIP3A maize leaf protein retains its biological activity following incorporation into five representative soil types (e.g., clay, sandy clay loam, silt loam, and the artificial soil as sandy loam). Based on insect bioassays performed with the black cutworm (BCW) *Agrotis ipsilon*, the DT50s (time to dissipation of 50% of initial bioactivity) for 58 and 14 µg VIP3A/g dry weight equivalent soil equaled five days or less for both treatment levels following a 3 - 12 day lag phase. Since the lag phase should not have been disregarded, DT50 for 58 µg VIP3A /g dry weight soil is =17 days.	Cry1F protein is expressed in minute quantities and is retained within the plant. Therefore, common modes of toxicity or routes of exposure are generally not relevant to consideration of the cumulative exposure to *Bacillus thuringiensis* Cry1F insect control protein. The product has demonstrated low toxicity to a large number of organisms listed in this table. In addition, the protein is not likely to be present in drinking water because the protein is deployed in minute quantities within the plant. The time-dependent loss in bioavailability of CrylF protein following incorporation into a typical maize-growing soil was determined under laboratory conditions (Halliday, 1998).The results of this study indicated that soil-applied CrylF protein exhibited a greater than 20-fold decline in biological activity over the 28-day test period. The estimated DT_{50} was 3.13 days. These results are consistent with those for CrylA(b) protein using essentially the same experimental design; a soil DT_{50} of 1.6 days was reported for the CrylA(b) protein.	In soils: Chlorpyrifos is moderately persistent with a half-life of usually 60 and 120 days, and a range from 2 wks - > 1 yr., depending on the soil type, climate, and other conditions. It was less persistent in soils with a higher pH (greater than 7.4). Soil half-life was not affected by soil texture or organic matter content. Adsorbed chlorpyrifos is subject to degradation by UV light, chemical hydrolysis and by soil microbes. When applied to moist soils, the volatility half-life was 45 to 163 hours, with 62 to 89% of the applied chlorpyrifos remaining on the soil after 36 hours. In another study, 2.6 and 9.3% of the chlorpyrifos applied to sand or silt loam soil remained after 30 days. Chlorpyrifos adsorbs strongly to soil particles and it is not readily soluble in water. It is therefore immobile in soils and unlikely to leach or to contaminate groundwater. TCP, the principal metabolite of chlorpyrifos, is moderately mobile and persistent in soils. In water: The concentration and persistence of chlorpyrifos will vary depending on the type of formulation. The increase in the concentration of insecticide is slower for granules and controlled release formulations in the water, but the resulting concentration persists longer . Volatilization is probably the primary route of loss of chlorpyrifos from water. Volatility half-lives of 3.5 and 20 days have been estimated for pond water. The photolysis half-life is 3 to 4 weeks during midsummer in the U.S. Research suggests that in water the rate at which it is hydrolyzed decreases by 2.5- to 3-fold with each 10 C drop in temperature. The rate of hydrolysis increases in alkaline waters. In water at pH 7.0 and 25˚C, it had a half-life of 35 to 78 days. In vegetation: Chlorpyrifos may be toxic to some plants. Residues remain on plant surfaces for ~ 10 to 14 days. This insecticide and its soil metabolites can accumulate in certain crops.	Permethrin is of low to moderate persistence in the soil environment, with reported half-lives of 30 to 38 days. Permethrin is readily broken down, or degraded, in most soils except organic types. Soil microorganisms play a large role in the degradation of permethrin in the soil. The addition of nutrients to soil may increase the degradation of permethrin. It has been observed that the availability of sodium and phosphorous decreases when permethrin is added to the soil. Permethrin is tightly bound by soils, especially by organic matter. Very little leaching of permethrin has been reported. It is not very mobile in a wide range of soil types. Because permethrin binds very strongly to soil particles and is nearly insoluble in water, it is not expected to leach or to contaminate groundwater. The results of one study near estuarine areas showed that permethrin had a half-life of less than 2.5 days. When exposed to sunlight, the half-life was 4.6 days. Permethrin degrades rapidly in water, although it can persist in sediments. Breakdown in vegetation: Permethrin is not phytotoxic, or poisonous, to most plants when it is used as directed. No incompatibility has been observed with permethrin on cultivated plants.

Avian toxicity	Nine-week old bobwhite quail were given a single oral dose of 2000 mg VIP3A-0198/kg body weight and observed for 14 days. The LC_{50} was > 2000 mg/kg body weight which is equiavalent to 400 mg VIP3A protein/kg body weight.	A summary value for acute toxicity for bobwhite quail chicks shows an LC_{50}>100,000 mg of grain from Cry1F corn/kg diet (the highest concentration tested). This is equivalent to 10% or 100,000 ppm of the diet being derived from Cry1F corn.	Chlorpyrifos is moderately to very highly toxic to birds. Its oral LD_{50} is 8.41 mg/kg in pheasants, 112 mg/kg in mallard ducks, 21.0 mg/kg in house sparrows, and 32 mg/kg in chickens. The LD_{50} for a granular product (15G) in bobwhite quail is 108 mg/kg. At 125 ppm, mallards laid significantly fewer eggs. There was no evidence of changes in weight gain, or in the number, weight, and quality of eggs produced by hens fed dietary levels of 50 ppm of chlorpyrifos.	Effects on birds: Permethrin is practically non-toxic to birds. The oral LD_{50} for the permethrin formulation, Pramex, is greater than 9900 mg/kg in mallard ducks, greater than 13,500 mg/kg in pheasants, and greater than 15,500 mg/kg in Japanese quail.
Aquatic Data	Catfish were fed VIP3A in diet for 30 days with no adverse effects. The LC_{50} was estimated by ELISA to be ≥7.1 µg VIP3A/g feed (7.1 ppm). In a 48-hour static renewal test, *Daphnia magna* (< 24 hours old) were fed VIP3A inbred corn pollen. The EC_{50} based on immobilization of Daphnids was >120 mg pollen/L.	There is no evidence for sensitivity of endangered aquatic species to Cry1F delta endotoxin. Low potential for exposure to Cry1F through drifting Cry1F maize pollen or other tissues derived from Cry1F maize and toxicity studies with aquatic invertebrates show very limited hazard for fish or invertebrates exposed to Cry1F. The measured effect level (EC_{50}) for the 48 hr. acute dietary toxicity study with *Daphnia magna* was greater than 100 mg Cry1F pollen/liter. This level is several fold higher than the estimated concentration of 1.25 µg Cry1F/liter from pollen drift into fresh water ponds.	Chlorpyrifos is very highly toxic to freshwater fish, aquatic invertebrates and estuarine and marine organisms. Cholinesterase inhibition was observed in acute toxicity tests of fish exposed to very low concentrations of this insecticide. Application of concentrations as low as 0.01 pounds of active ingredient per acre may cause fish and aquatic invertebrate deaths. Chlorpyrifos toxicity to fish may be related to water temperature. The 96-hour LC_{50} for chlorpyrifos is 0.009 mg/L in maturerainbow trout, 0.098 mg/L in lake trout, 0.806 mg/L in goldfish, 0.01 mg/L in bluegill, and 0.331 mg/L in fathead minnow]. Chlorpyrifos accumulates in the tissues of aquatic organisms. Studies involving continuous exposure of fish during the embryonic through fry stages have shown bioconcentration values of 58 to 5100. Due to its high acute toxicity and its persistence in sediments, chlorpyrifos may represent a hazard to sea bottom dwellers. Smaller organisms appear to be more sensitive than larger ones .	Effects on aquatic organisms: Aquatic ecosystems are particularly vulnerable to the impact of permethrin. A fragile balance exists between the quality and quantity of insects and other invertebrates that serve as fish food. The 48-hour LC_{50} for rainbow trout is 0.0125 mg/L for 24 hours, and 0.0054 mg/L for 48 hours. As a group, synthetic pyrethroids were toxic to all estuarine species tested. They had a 96-hour LC_{50} of less than or equal to 0.0078 mg/L for these species. The compound has a low to moderate potential to accumulate in these organisms.
Non-target and beneficial insects	The LD_{50} for lady beetles (*Coleomegilla maculata*) fed 5% VIP3A pollen in the diet for 21 days was >7.24 ppm. VIP3A corn pollen fed to 2 - 4 day old adult green lacewings (*Chrysoperla carnea*) in diet for 13 days did not have a significant effect on adult survival or fitness. Treatments consisted of 3.5 g of corn pollen (144.8 µg of VIP3A protein per gram of pollen) added to 19.6 g of standard lacewing diet. Submitted data shows that lacewings were fed 15% of their diet as VIP3A pollen; therefore, the LC_{50} is >21.7 ppm.	Results indicated that Cry1F delta endotoxin (produced microbially) has an acute LC_{50} greater than 320 µg Cry 1F/g diet for parasitic Hymenoptera (*Nasonia vitripennis*), and an acute LC_{50} greater than 480 µg Cry 1F/g diet for green lacewing (*Chrysoperla carnea*) and lady bird beetle (*Hippodamia convergens*). These concentrations are several fold higher than the upper bound estimate of 32 µg Cry 1F/g pollen derived from line 1507 corn, and indicate low potential for toxicity due to exposure.	Aquatic and general agricultural uses of chlorpyrifos pose a serious hazard to wildlife and honeybees.	Effects on other organisms: Permethrin is toxic to wildlife. It should not be applied, or allowed to drift, to crops or weeds in which active foraging takes place. The International Organization for Biological Control tested the acute toxicity of permethrin to 13 species of beneficial arthropods and found that permethrin caused 99 percent mortality of 12 of the species, and over 80 percent mortality of the other. Effects were persistent, lasting over 30 days. Sublethal doses also impact beneficial arthropods: permethrin inhibited the emergence of a parasitoid wasp from eggs of the rice moth *Corcyra cephalonica* and disrupted the foraging pattern of another parasitoid wasp as it searched for its aphid prey.

Honeybee toxicity	Exposure of larval honey bees to a 2 mg dose of corn pollen containing the equivalent of 168 µg of VIP3A protein did not have a significant effect on bee development or survival.	A petition by Dow-Mycogen to deregulate Cry1F maize contains details of this analysis in a CBI appendix, and the petition summary indicates an acute dietary toxicity (honeybees) LD_{50}> 640 ng Cry1F/larvae	Aquatic and general agricultural uses of chlorpyrifos pose a serious hazard to honeybees.	Permethrin is extremely toxic to bees. Severe losses may be expected if bees are present at treatment time, or within a day thereafter.
Non-target soil organisms	Exposure to VIP3A corn leaf tissue did not have a significant effect on Collembola survival and reproduction. Dietary LC_{50}s were >43.1 µg/g dry weight, the highest levels tested. At the highest concentration tested (1000 mg VIP3A Maize Leaf Protein/kg soil = 3.9 mg VIP3A/kg soil), the test substance had no adverse effects on the earthworm *Eisenia foetida*.	A 28-day study to determine the chronic effects of microbially-derived CrylF protein on survival and reproduction of Collembola was conducted with three treatment levels of the CrylF test substance (0.63, 3.1, and 12.5 mg/kg of test diet). At the conclusion of the test, there was less than 10% mortality associated with exposure to either the CrylF protein test substance or the assay control. Reproduction of Collembola was not significantly affected by exposure to the test substance when compared to the assay control. No mortality and no reduction in the number of progeny was observed following exposure to the test materials for 28 days. The results of this study indicate Collembola were not affected by chronic exposure to CrylF at treatment levels exceeding those expected to be found in maize fields based on the calculated worst-case, post-harvest exposure estimates of 0.350 mg Cry1F protein/kg of whole plant material at senescence or 0.063 mg Cry1F protein/kg dry soil. Acute toxicity for earthworm was established by exposure to microbially-produced Cry1F protein in soil. The LC_{50} was > 2.5 mg Cry1F/kg dry soil. This concentration is also considerably higher than the worst-case estimate of Cry1F post-harvest exposure in the soil.	Data not found in sources consulted.	Data not found in sources consulted.
EPA toxicity class (Class I -highly toxic to Class IV-relatively non-toxic)	Not assigned.	Not assigned.	Chlorpyrifos is toxicity class II - moderately toxic. Products containing chlorpyrifos bear the Signal Word WARNING or CAUTION, depending on the toxicity of the formulation. It is classified as a General Use Pesticide (GUP). The EPA has established a 24-hour reentry interval for crop areas treated with emulsifiable concentrate or wettable powder formulations of chlorpyrifos unless workers wear protective clothing.	Permethrin is a moderately to practically non-toxic pesticide in EPA toxicity class II or III, depending on the formulation. Formulations are placed in class II due to their potential to cause eye and skin irritation. Products containing permethrin must bear the Signal Word WARNING or CAUTION, depending on the toxicity of the particular formulation. All products for agricultural uses (except livestock and premises uses) are Restricted Use Pesticides (RUPs) because of their possible adverse effects on aquatic organisms.
EDF - Integrated Environmental Rankings[5] - Combined human & ecological scores	not ranked	not ranked	50 to 75%	0 to 25%

Mammalian toxicity	Toxicology studies conducted to determine the toxicity of VIP3A insect control protein demonstrated that the protein has very low toxicity. A single dose of 5000 mg/kg body weight (equivalent to 18 mg VIP3A/kg body weight) of VIP3Aextracted from plants was administered to 4 - 6 week old mice via gavage resulted in no effects after 14 days. A single dose of 5050 mg/kg body weight (equivalent to 1616 mg VIP3A/kg body weight) microbially derived VIP3A protein fed to mice via gavage also resulted in no effects after 14 days. Proteins that are not readily degraded in the gastric environment mayhave increased potential to elicit toxicity and stimulate an allergic response if they are not subsequently digested in the intestine. In the presence of SGF at the standard pepsin concentration, VIP3A from *E. coli* was degraded at time zero to peptides of less than 14,000 molecular weight. After 2 min of incubation, this lower molecular weight material was no longer visible. In another study, VIP3A protein as produced in either Pacha-derived maize or recombinanat *E.coli* was completely degraded in DIF following a pre-incubation in SGF without pepsin which indicates that exposure of VIP3A to gastric pH, even in the absence of pepsin, prior to exposure to intestinal fluid will allow the protein to be rapidly digested in mammalian intestinal environment.	Toxicology studies conducted to determine the toxicity of Cry1F insect control protein demonstrated that the protein has very low toxicity. In an acute oral toxicity study in the mouse, the estimated acute LD_{50} by gavage was determined to be >5,050 mg of the microbially produced test substance containing 576 mg Cry1F/kg body weight. This dose is 12,190 times greater than the estimated 95th percentile for human dietary exposure to Cry1F protein resulting from consumption of foods derived from Cry1F protected corn. In an in vitro study, Cry1F protein was rapidly and extensively degraded in simulated gastric conditions in the presence of pepsin. This indicates that the potential for adverse health effects from chronic exposure is virtually nonexistent. A search of relevant databases indicated that the amino acid sequence of the Cry1F protein exhibits no significant homology to the sequences of known allergens or protein toxins. Thus, Cry1F is highly unlikely to exhibit an allergic response. Collectively, the available data on Cry1F protein along with the safe use history of microbial *Bacillus thuringiensis* products establishes the safety of the plant pesticide *Bacillus thuringiensis* subspecies *aizawai* Cry1F insect control protein and the genetic material necessary for its production in all raw agricultural commodities.	**Acute toxicity:** Chlorpyrifos is moderately toxic to humans. Poisoning may affect the central nervous system, the cardiovascular system, and the respiratory system. It is also a skin and eye irritant. Studies in humans suggest that skin absorption of chlorpyrifos is limited. The oral LD_{50} for chlorpyrifos in rats is 95 to 270mg/kg, 60 mg/kg in mice, 1000 mg/kg in rabbits, 32 mg/kg in chickens, 500 to 504 mg/kg in guinea pigs, and 800 mg/kg in sheep. The dermal LD_{50} is greater than 2000 mg/kg in rats, and 1000 to 2000 mg/kg in rabbits. The 4-hour inhalation LC50 for chlorpyrifos in rats is greater than 0.2 mg/L. **Chronic toxicity:** Repeated or prolonged exposure to organophosphates may result in the same effects as acute exposure including the delayed symptoms. Human volunteers who ingested for 4 weeks 0.1mg/kg/day of chlorpyrifos showed significant plasma cholinesterase inhibition. **Reproductive effects:** Current evidence indicates that chlorpyrifos does not adversely affect reproduction. No effects were seen in 2 studies where animals were tested at doses up to 1.2 mg/kg/day. **Teratogenic effects:** Available evidence suggests that chorpyrifos is not teratogenic. Three studies in pregnant rats or mice indicate that no significant teratogenic effects were seen at doses up to 25 mg/kg/day for 10 days. **Mutagenic effects:** No evidence was found in any of four tests performed that chlorpyrifos is mutagenic. **Carcinogenic effects:** There is no evidence that chlorpyrifos is carcinogenic. There was noincrease in the incidence of tumors when rats were fed 10 mg/kg/day for 104 weeks. **Fate in humans and animals:** Chlorpyrifos is readily absorbed into the bloodstream through the gastro-intestinal tract if it is ingested, through the lungs if it is inhaled, or through the skin if there is dermal exposure. In humans, chlorpyrifos and its principal metabolites are eliminated rapidly. After a single oral dose, the half-life of chlorpyrifos in the blood appears to be about 1 day.	**Acute toxicity**: Permethrin is moderately to practically non-toxic via the oral route. Via the dermal route, it is slightly toxic, with a reported dermal LD_{50} in rats of over 4000 mg/kg, and in rabbits of greater 2000 mg/kg. Permethrin caused mild irritation of both the intact and abraded skin of rabbits. It also caused conjunctivitis when it was applied to the eyes. The 4-hour inhalation LC_{50} for rats was greater than 23.5 mg/L, indicating practically no inhalation toxicity. **Chronic toxicity**: No adverse effects were observed in dogs fed permethrin at doses of 5 mg/kg/day for 90 days. Rats fed 150 mg/kg/day for 6 months showed a slight increase in liver weights. **Reproductive effects**: The fertility of female rats was affected when they received very high oral doses of 250 mg/kg/day of permethrin during the 6th to 15th day of pregnancy. It is not likely that reproductive effects will be seen in humans under normal circumstances. **Teratogenic effects**: Permethrin is reported to show no teratogenic activity. **Mutagenic effects**: Permethrin is reported to show no mutagenic activity. **Carcinogenic effects**: The evidence regarding the carcinogenicity of permethrin is inconclusive. **Organ toxicity**: Permethrin is suspected of causing liver enlargement and nerve damage. **Fate in humans and animals**: Permethrin is efficiently metabolized by mammalian livers. Breakdown products, or "metabolites," of permethrin are quickly excreted and do not persist significantly in body tissues. Permethrin may persist in fatty tissues, with half-lives of 4 to 5 days in brain and body fat.

[1] Bt VIP3A data summary. Petition for Determination of non-regulated status of Syngenta Seeds VIP3A cotton Event COT102. This petition is assigned APHIS petition number 03-155-01p.

[2] B.t Cry1F data summary. Petition for Determination of non-regulated status B.t. Cry1F insect-resistant glufosinate-tolerant maize line 1507 (2000) Shanahan, D. and Stauffer, C. Mycogen Seeds, Dow Agrisciences and Pioneer Hi-Bred Intl. Inc. (2000). This petition is assigned APHIS petition number 00-136-01p. The mammalian toxicity profile is derived from the petitioner summary of the pesticide petition to establish an exemption from the requirement of a tolerance for the plant-pesticide *Bacillus thuringiensis* Cry1F protein and the genetic material necessary for its production in plants in or on all food commodities as it appears in the Federal Register: June 15, 2000 (Volume 65, Number 116), pp 37545-37547.

[3]Chlorpyrifos Data: Pesticide Information Profiles, EXTOXNET Extension Toxicology Network. Revised June 1996. http://ace.orst.edu/cgi-bin/mfs/01/pips/chlorpyr.htm.
Chemical Fact Sheet for : Chlorpyrifos, Fact Sheet Number: 37, Date Issued: September 30, 1984 available at
http://pmep.cce.cornell.edu/profiles/insect-mite/cadusafos-cyromazine/chlorpyrifos/index.html

[4] Permethrin Data: Pesticide Information Profiles, EXTOXNET Extension Toxicology Network. Revised June 1996. http://ace.orst.edu/cgi-bin/mfs/01/pips/permethr.htm?8#mfs; Insecticide Fact Sheet, Coalition for Alternatives to Pesticides/NCAP, P.O.Box 1393, Eugene, Oregon,. J. of Pesticide Reform, Summer, 1998, v. 18, no. 2141. http://www.safe2use.com/poisons-pesticides/pesticides/permethrin/cox.htm

[5]For EDF rankings, Environmental Defense Fund. *http://www.scorecard.org/chemical-profiles/*

Appendix D. Summary table of data submitted with the petition in support of nonregulated status for Syngenta Event COT102.

Molecular Characterization Data	**Figure/Table and Page in Petition**
Plasmid map of pCOT1	Fig. 3.1, p. 34
Southern blot for intactness of insert, *vip3A(a)* coding region and promoter, and copy number	Fig. 3.2a, p.37; Fig. 3.2b, p.38; Fig. 3.3a, p. 40
Southern blot for intactness of *aph4* coding region and promoter, and copy number	Fig. 3.4a, p. 44; Fig. 3.5a, p. 46
Southern blot for lack of plasmid backbone sequences	Fig. 3.6a, p. 50; Fig. 3.7a, p. 52; Fig. 3.8a, p. 54; Fig. 3.9a, p. 56
Vip3A protein level in various plant tissues collected from multiple field sites during the growing season	Table 6.1, p. 105; Table 6.2, p. 106; Table 6.5, p. 109; Table 6.6, p. 110
APH4 protein level in various plant tissues collected from multiple field sites during the growing season	Table 6.3, p. 107; Table 6.4, p. 108; Table 6.5, p. 109; Table 6.6, p. 110
Mendelian inheritance and stability	Table 3.4, p 58
Characterization of VIP3A protein produced in COT102-derived Cotton and Comparison with VIP3A Protein Expressed in Both Maize (Corn) Derived from Event PACHA and Recombinant *E. coli*	petition appendix 20
Characterization of VIP3A Protein Produced in PACHA-Derived Maize (Corn) and comparison with VIP3A Protein Expressed in Recombinant *E. coli*	petition appendix 22
Agronomic Characterization Data	
Disease susceptibility evaluation	Table 4.1, p. 62-63; p. 87
Insect susceptibility evaluation	Tables 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8,4.9, 4.10, 4.11, pages 64-72; p. 87.
Agronomic parameters, yield, plant stand, plant height, number of fruiting branches, number of nodes, height to node ratio, fiber quality, node of first fruiting branch	Tables 4.13, p.76; Table 4.14, p.77; Table 4.15, p.78; Fig. 4.2, p.81; Fig. 4.3, p.81; Fig. 4.4, p.82; Fig. 4.5, p.83; Fig. 4.6, p.84; Table 4.17, p.84; Fig. 4.7, p.85; Fig. 4.8, p.85; Fig. 4.9, p.86; Fig. 4.10, p.86; Fig. 4.11, p.86
Toxin and Antinutrients	Tables 5.16 through 5.18, p.96; Tables 5.19 and 5.20, p.97, petition appendix 10
Plant stand and seed germination	Table 4.12, p. 76; Table 4.16, p. 79; Table 4.18, p. 87
Plant tissue compositional analyses	Tables 5.1 through 5.15, pages 90 – 94

Non-target data	

Non-target data	petition Tables 1.2; 1.3; p.124-151
Environmental Safety Assessment of *Bacillus thuringiensis* VIP3A Protein and VIP3A cotton Event COT102 to Non-target Organisms	petition appendix 15
Acute toxicity with VIP3A in Bobwhite Quail	petition appendix 6
Acute toxicity with VIP3A in Daphnids	petition appendix 7
Acute toxicity with VIP3A in Pink-Spotted Lady Beetle	petition appendix 9
Toxicity with VIP3A & Cru1Ab in Collembola	petition appendix 18
Toxicity with VIP3A in Green Lacewing	petition appendix 16
Toxicity with VIP3A in Honeybee development	petition appendix 17
Impact of Transgenic Lepidopteran-Resistant VIP3A Field Corn (Maize) on Honey Bee Colonies in a Semi-field Setting	petition appendix 21
Acute toxicity with VIP3A in Earthworms	petition appendix 8
Biological Activity of VIP3A in Various Soils	petition appendix 19
Summary of Mammalian Safety Data for VIP3A and APH4 proteins	petition appendix 11
Acute toxicity with VIP3A in mice	petition appendices 2, 3, 4, 5
Acute toxicity with APH4 in mouse	petition appendix 14
In vitro digestibility of VIP3A and APH4 proteins under simulated mammalian gastric and intestinal conditions	petition appendices 12 & 13