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Alexander B. Grannis
Commissioner

May 19, 2009

DELIVERY CONFIRMATION

Mr. Lawrence Zeph
Syngenta Seeds, Inc.
3054 E. Cornwallis Road
Research Triangle Park, North Carolina 27709

Dear Mr. Zeph:

RE: Registration of Bt11 x MIR162 Corn (EPA Reg. No. 67979-12), Bt11 x MIR162 x MIR604 Corn (EPA Reg. No. 67979-13) and MIR162 Maize (EPA Reg. No. 67979-14 Containing the New Active Ingredient *Bacillus thuringiensis* Vip3Aa20 Insecticidal Protein, and the Genetic Material Necessary for its Production (Active Ingredient Chemical Code 006599)

The New York State Department of Environmental Conservation (Department) has completed the review of your application and data package, received on March 10, 2009, in support of the registration of Bt11 x MIR162 Corn (EPA Reg. No. 67979-12), Bt11 x MIR162 x MIR604 Corn (EPA Reg. No. 67979-13) and MIR162 Maize (EPA Reg. No. 67979-14. Bt11 x MIR162, Bt11 x MIR162 x MIR604, and MIR162 Maize are defined as Plant Incorporated protectants (PIPs), meaning that a pesticidal gene has been introduced into the plant's genetic material.

Bt11 x MIR162 Corn (EPA Reg. No. 67979-12) contains 0.00140-0.00201% *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production, and 0.0068-0.0087% *Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production. It is intended for use only in the production of corn with the goal of providing protection from European corn borer, Southwestern corn borer, Southern cornstalk borer, corn earworm, fall armyworm, beet armyworm, black cutworm, western bean cutworm, sugarcane borer and common stalk borer.

Bt11 x MIR162 x MIR604 Corn (EPA Reg. No. 67979-13) contains 0.0014-0.0017% *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production, 0.0057-0.0088% *Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production, and 0.0013-0.0021% *Bacillus thuringiensis* mCry3A protein and the genetic material necessary for its production. It is intended for use only in the production of corn with the goal of providing protection from European corn borer, Southwestern corn borer, Southern cornstalk borer, corn earworm, fall armyworm, beet armyworm, black cutworm, western bean cutworm, sugarcane borer and common stalk borer, Western corn rootworm, Mexican corn rootworm and common stalk borer.

MIR604 Corn (EPA Reg. No. 67979-143) contains 0.00167-0.00751% *Bacillus thuringiensis*

Vip3Aa20 insecticidal protein and the genetic material necessary for its production. It is intended for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed on up to a total of 20,000 acres per county and up to a combined United States total of 30,000 acres per year.

The subject application and data package were deemed complete for purposes of technical review on May 5, 2009. Pursuant to the review time frame specified in Environmental Conservation Law (ECL) §33-0704.2, a registration decision date of October 5, 2009 was established. Due to the substantially similar nature of this active ingredient to subspecies previously registered by the Department, it was determined that neither a human health technical review nor an environmental fate technical review would be performed for this application. Hence, only an ecological effects technical review was undertaken. Shown below is the technical review produced by the Department's Bureau of Habitat

Ecological Effects Technical Review:

Ecological Risk Assessment of Transgenic Corn with the Insecticidal Protein Vip3Aa20:

1. BACKGROUND AND CHEMICAL DESCRIPTION

The soil bacteria, *Bacillus thuringiensis* has long been known to produce crystalline (CRY) proteins known as δ-endotoxins when the bacterium forms a spore. These CRY proteins are toxic to insects. When an insect consumes the spore, the CRY protein is solubilized and activated. They then bind to aminopeptidase-N (APN) and cadherin-like proteins[1] in the midgut wall of the insect, and form a pore-like structure through the midgut wall, effectively perforating it and releasing the midgut contents into the insect's body cavity, where it eventually dies of septicemia.

Several pesticide products have been produced using the spores harvested from the bacteria as the active ingredient. In the past 10 years, the genes for the production of the CRY proteins have been spliced into the DNA for crops such as corn, cotton, and soybeans, producing a class of products known as Plant Incorporated Protectants (PIPs). The Bureau of Habitat (BOH) has reviewed several corn PIP products consisting of genetically incorporated CRY proteins that are toxic to various lepidopteran and coleopteran pests.

The purpose of this review is to examine a new PIP that has not been reviewed previously, the Vip3Aa20 insecticidal protein.

VIP is an acronym for Vegetative Insecticidal Protein. Like CRY proteins, VIPs are also produced by *Bacillus thuringiensis*, however, they are produced during the vegetative stage of the bacterium's life cycle, not during the spore stage. They are also released as soluble proteins and not as crystals. VIPs do not bind to APN and cadherin-like receptors, although, like CRY proteins, they do form tube-like pores in the insect's midgut, thus perforating it.

The inclusion of a second, different PIP is important for resistance management. If a target insect survives exposure to a CRY protein because of genetic resistance to the toxin, that trait can be passed down to future generations, and strains of resistant insects might develop. However, if a plant incorporates two dissimilar PIPs, there is a much smaller likelihood that a target insect would be resistant to both PIPs. That is, if it can survive exposure to the CRY protein, it still might succumb to the VIP protein (and vice versa). Thus, the likelihood of the development of genetic resistance to either protein is diminished.

Three specific products are proposed for registration in New York. MIR162 Maize incorporates only the Vip3Aa20 insecticidal protein. It is not being produced as a consumer product. Rather, it is being produced only for breeding purposes, agronomic testing, and producing other hybrids.

BT11 x MIR162 Corn is a hybrid of the MIR162 corn with the Vip3Aa20 protein and BT11

corn with the CRY1Ab protein[2]. It provides protection for corn primarily against lepidopteran pests such as the European corn borer, Southwestern corn borer, Southern cornstalk borer, corn earworm, fall armyworm, and the black cutworm.

BT11 x MIR162 x MIR604 Corn is a hybrid of the MIR162 corn with the Vip3Aa20 protein, BT11 corn with the CRY1Ab protein, and the MIR604 corn with the mCRY3A protein. The inclusion of the mCRY3A protein allows for the protection of corn against both lepidopteran and coleopteran pests of corn, which include the European corn borer, Southwestern corn borer, Southern cornstalk borer, corn earworm, fall armyworm, black cutworm, Western corn rootworm, Northern corn rootworm and Mexican corn rootworm.

Both the CRY1a and CRY3a PIPs have been reviewed by Bureau of Habitat. This review will focus only on the Vip3Aa20 protein as found in MIR162 corn.

2. ASSESSMENT OF TOXICITY

The toxicity of the Vip3Aa20 protein was tested with mammals, birds, and numerous non-target insects, including honeybees. It exhibited the same lack of toxicity to non-target organisms that the CRY1 and CRY3 proteins exhibited. The results of toxicity studies are summarized in Table 1.

Table 1. Toxicity of Vip3A insecticidal BT protein to non-target organisms as found in MIR162 corn.

Organism	**Test**	**Results/Comments**
Bobwhite quail	Single oral dose (gavage)	No adverse effects to a single oral dose of 400 mg/kg bw. NOEL was 400 mg/kg and the LD_{50} was >400 mg/kg
Channel catfish	30 day feeding study; Vip3A protein incorporated into fish food	No adverse effects noted; NOEC (in food) was 7.10 ug/g and the LC_{50} was >7.10 ug/g
Daphnia magna	48 hour static renewal bioassay	Corn pollen containing 10.1 ug Vip3A protein was suspended in water at a concentration of 120 mg pollen/L. No adverse effects noted, and the LC_{50} was found to be >10.1ug Vip3A/L. However, U.S. EPA guidelines call for a 21 day study so this study was classified as unacceptable.
Minute pirate/ insidious flower bug, *Orius insidiosus*	21 day feeding study, Vip3A protein incorporated into meat diet	No adverse effects noted. Vip3A NOEC was 7.25 mg/g; LC_{50} was >7.25 mg/g
Pink spotted lady beetle	21 day feeding study, diet containing MIR162 pollen grains	No adverse effects noted. Vip3A NOEC was 7.24 ug/g pollen; LC_{50} was >7.24 ug/g pollen
Seven-spotted ladybird beetle	15 day feeding study, Vip3A protein incorporated into sucrose solution	No adverse effects noted. Vip3A NOEC was 7250 ug/g diet; LC_{50} was >7250 ug/g diet
Green lacewing	14 day feeding study, Vip3A protein incorporated into meat diet	No adverse effects noted. Vip3A NOEC was 7250 ug/g diet; LC_{50} was >7250 ug/g diet
Rove beetle	35 day feeding study. Vip3A protein incorporated into meat	No adverse effects noted. Vip3A NOEC was 595.3 ug/g diet; LC_{50} was

Organism	Test	Results/Comments
	diet. Reproductive effects also measured by counting the number of second-generation adult beetles emerging from parasitized onion fly larvae	>595.3 ug/g diet.
Honeybee	Oral ingestion	Bees were provided Vip3A incorporated in sucrose solution at a maximum concentration of 500 ug/g solution, one liter per hive. Hives were then observed for 24 days. Vip3A did not adversely affect brood development, exposed workers, or hive condition. Vip3A NOEC was 500 ug/g sucrose solution; LC_{50} was >500 ug/g sucrose solution.
Earthworm	14 day exposure in soil	No mortality, or differences in body weights were noted. Vip3A NOEC was 3.60 ug/g soil; LC_{50} was >3.60o ug/g soil.

A condition of U.S. EPA registration is a 7-14 day *Daphnia magna* study, or as an alternative, a dietary study of the effects of Vip3Aa20 on an aquatic invertebrate that represents the functional group of a leaf shredder in headwater streams, which is due to the U.S. EPA in November 2009.

In their review, U.S. EPA reported that there may have been a synergistic effect between MIR162 and MIR604 (i.e., between the Vip3Aa20 and mCRY3A insecticidal proteins) in the control of corn rootworm, but the sample sizes were two small to reach a definite conclusion. No other possible synergism was noted with other target organisms.

3. EXPOSURE ASSESSMENT

The concentrations of Vip3Aa20 in different tissues of the three different corn hybrids are listed in Table 2.

Table 2. Highest concentration of Vip3Aa20 in different tissues of MIR162 corn and two corn hybrids in ug/g.

	Hybrid/variety		
Tissue	**MIR162 Corn**	**BT11 x MIR162**	**BT11 x MIR162 x MIR604**
Leaves	56.56	259	214
Kernels	30.90	164.7	102.1
Roots	6.20	65.8	38.4
Pollen	47.85	95.9	174
Whole plant	24.62	87.5	86.5

The persistence of Vip3Aa20 was tested in five different soils. The half-life due to microbial degradation was 6.0 – 12.6 days. Vip3Aa20 was also degraded rapidly in simulated gastric juices.

The most likely routes of exposure for non-target birds and mammals is direct feeding on corn with Vip3Aa20 incorporated as a PIP. Aquatic organisms are likely to be exposed through the drift of corn pollen to adjacent waters. Non-target insects are likely to be exposed through feeding on corn plant tissues or by consuming pollen that blown by wind away from cornfields.

4. RISK ASSESSMENT

Risk is a product of both toxicity and exposure. Highly toxic compounds present little risk if fish and wildlife are not exposed to them. Conversely, there can be significant risk from substances that are not necessarily highly toxic, if there is considerable exposure.

In regards to VIP3Aa20 insecticidal protein, the risks to non-target organisms are slight both because toxicity and exposure are very low. Like the CRY proteins, Vip3Aa20 appears to be toxic to a very small, specific group of target organisms, primarily in the orders Lepidoptera and Coleoptera. Earlier literature reviewed suggested that insecticidal proteins are only efficacious against insects with alkaline gut systems. The suite of non-target organisms tested showed no effects, even when exposed to concentrations 10x higher than they would be likely to encounter in actual field conditions.

Exposure to corn pollen with VIP3Aa20 is also likely to be low. Corn pollen is heavy, and downwind concentrations of corn pollen drop off rapidly as distance increases from the source (i.e., cornfield).

The U.S. EPA considered the potential for MIR162 corn to impact rare, threatened, or endangered species. The only such species likely to have any risk from Vip3Aa20 would be the Karner blue butterfly. However, the time that Karner blue larvae are actively foraging does not overlap with the short period of time that corn pollen is produced and released.

Earlier assessments of CRY PIPs examined the potential for transgenic genes to be transferred to wild plant populations. This likelihood was considered to be low because there are no naturally-occurring relatives of corn in the wild that can crossbreed with corn present in the United States. Some related plants occur in Mexico and Central America, but these do not naturally crossbreed with corn, and on rare occasion when they have, or when they have been artificially crossbred with corn in the lab, the surviving offspring were sterile. This assessment of the likelihood of gene transfer to wild plant populations is equally valid and applicable Vip3Aa20 PIPs as well as CRY PIPs.

5. CONCLUSION

The Vip3Aa20 insecticidal protein is considerably different than the CRY1A and CRY3A insecticidal proteins that have been reviewed before. Both types of protein originate with the soil bacterium *Bacillus thuringiensis*. However, CRY proteins are crystals that are produced during the bacterium's spore stage, and the VIP protein is a soluble liquid protein that is produced during the vegetative stage of the bacterium's life cycle. CRY proteins bind to APN and/or cadherin-like receptors in the insect's midgut wall. VIP proteins bind to a different set of receptors. Despite these differences, the toxicity of the VIP proteins appear to be about the same as the toxicity of the CRY proteins, that is, highly specific for a few lepidopteran or coleopteran groups, and generally non-toxic to most other organisms.

There is likely to be little risk to fish and wildlife from the use of Vip3Aa20 as a PIP in corn. A significant benefit of employing Vip3Aa20 as a PIP in corn hybridized with other, CRY PIPs is that it decreases the potential for development of insect resistance to *Bt*-based insecticidal proteins.

REGISTRATION SUMMARY

The Department has registered **Bt11 x MIR162 Corn (EPA Reg. No. 67979-12), Bt11 x**

MIR162 x MIR604 Corn (EPA Reg. No. 67979-13) and MIR162 Maize (EPA Reg. No. 67979-14 for use in New York State.

Enclosed for your records is a copy of each stamped "Accepted for Registration" label and a New York State Certificate of Pesticide Registration for Bt11 x MIR162 Corn, Bt11 x MIR162 x MIR604 Corn and MIR162 Maize. Please note that the submission of *Bacillus thuringiensis* Vip3Aa20 Insecticidal Protein**,** and the genetic material necessary for its production whose labeled uses are likely to increase the potential for significant impact to humans, non-target organisms, or the environment, would constitute a major change in labeled use pattern. Such an application must be accompanied by a new application fee and meet the requirements listed in Appendix 1.B of "New York State Pesticide product Registration Procedures" (April 2009). Such information, as well as forms, can be accessed at our website as listed in our letterhead.

If you have any questions, please contact Luanne Whitbeck, of our Pesticide Product Registration Section, at (518) 402-8768.

Sincerely,

Maureen P. Serafini

Maureen P. Serafini
Director
Bureau of Pesticides Management

Enclosures

ecc: R. Mungari, NYSA&M
W. Smith, Cornell University, PSUR