UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES,
AND
TOXIC SUBSTANCES

~~CONFIDENTIAL BUSINESS INFORMATION~~

MEMORANDUM

Date: April 2, 2008

DP Barcode: 345911, 345915
Decision: 379488, 379490

SUBJECT: Review of Public Interest Document for Bt11xMIR162xMIR604 Maize (Syngenta) [EPA Reg. No. 67979-RG, MRID 471378-19, including the efficacy and IRM chapters (partial review) submitted separately, MRIDs 471530-01, 471530-02, 471530-03, 471530-04, 471531-01, 471531-02, 471531-03, 471372-12, respectively]

TO: Mike Mendelsohn, Regulatory Action Leader
Microbial Pesticide Branch
Biopesticides and Pollution Prevention Division (7511P)

FROM: Jeannette Martinez, Biologist
Microbial Pesticide Branch
Biopesticides and Pollution Prevention Division (7511P)
4/2/08

REVIEWED BY: Alan Reynolds, Entomologist
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4/2/08

ACTION REQUESTED:

BPPD[1] has been asked to review the public interest document (PID) for Bt11xMIR162xMIR604 Maize including the efficacy chapter and parts of the IRM chapter as they relate to the PID submitted by Syngenta.

[1] The use of BPPD in this review refers to the BPPD IRM and benefits team.

CONCLUSION AND RECOMMENDATIONS

To grant a conditional registration under Section 3(c)(7)(C) of FIFRA, EPA must determine that such conditional registration will, *inter alia*, be in the public interest. EPA determines whether conditional registration of a pesticide is in the public interest in accordance with the criteria set forth at 51 Fed. Reg. 7628 (*Conditional Registration of New Pesticides*, March 5 1986). On the basis of analysis utilizing these criteria, EPA concludes that the use of MIR162 Maize (and its stacked products) (Reg. No. 67979-RG) will be in the public interest because it results in direct and indirect human and environmental health benefits by providing growers with an additional choice of *Bt* corn product and the potential to extend the useful life of *Bt* corn technology generally due to the Vip3A proteins novel mode of action and low likelihood of cross-resistance with other *Bt* Cry proteins.

Public Interest

Syngenta's claims that "Bt11xMIR162xMIR604 is expected to provide unsurpassed control of target pests" and that the products "broad-lepidopteran control can potentially result in better performance than competitor offerings". BPPD notes that these statements are unverified assumptions.

BPPD concludes that MIR162, Bt11xMIR162, and Bt11xMIR162xMIR604 maize are expected to provide the public interest benefits shared by other corn PIPs already registered by the Agency. Specifically the stacked product would provide a new tool for farmers who face challenges of protecting corn crops from lepidopteran as well as coleopteran pest damage. In addition, the stacked product can be expected to prolong the life-time of other corn PIPs due to Vip3A having a novel mode of action.

A more detailed analysis of benefits derived from human health and environment and IRM will follow in sections 4 and 5.

Efficacy

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

There are indications from the Illinois data (ECB and CEW efficacy studies) that stacking the traits Bt11, MIR162, MIR604, and GA21 maize together may produce different efficacy results than for Bt11 plants alone. Whether this is due to synergistic effects between the toxins or environmental effects is unclear, and the registrant did not provide an explanation for the results. In future submissions of efficacy studies, BPPD requests that Syngenta address such differences by supplying possible explanations. However, BPPD notes that both the stacked and single trait containing products appear to provide good protection against ECB and CEW.

The data support that the stack containing Bt11, MIR162, MIR604 and GA21 traits provides good efficacy results against FAW.

The data support that the stack containing Bt11, MIR162, MIR604 and GA21 traits produces reasonably good efficacy against WCRW.

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

Grower Benefits

BPPD focuses on the benefits from MIR162, Bt11xMIR162, and Bt11xMIR162xMIR604 only and not on the potential economic benefits of these products containing the GA21 trait. It may be that additional benefits are derived from an herbicide tolerant trait in MIR162, Bt11xMIR162, and Bt11xMIR162xMIR604. On the other hand, such a trait could also increase the risk of weed resistance.

BPPD finds that MIR162, Bt11xMIR162, and Bt11xMIR162xMIR604 will likely have similar general economic grower's benefits of already registered corn PIPs (Bt11 and MIR604) as described by the Agency in the 2001 *Bt* crop reassessment and MIR604 BRAD. The Agency's summary of these benefits can be accessed online at http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm and (http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006509.pdf). In addition, Bt11xMIR162xMIR604 maize will provide further benefits by controlling corn root worm as well as several lepidopteran pests.

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

Human Health and Environmental Benefits

EPA's review of human health and environmental data is pending. Syngenta claims that there is no reason for concern with respect to hazard or allergenicity for the proteins expressed in MIR162, Bt11xMIR162, and Bt11xMIR162xMIR604. From the knowledge of other PIPs, BPPD expects the results for human health and environment to be similar and not to pose a problem. However, BPPD will confirm this upon completion of its review of Syngenta's data.

Insect Resistance Management Benefits

BPPD concludes that MIR162, Bt11xMIR162, and Bt11xMIR162xMIR604 have the following benefits: 1) high dose against FAW, ≥ near high-dose against CEW, and ≥ near high-dose against ECB, 2) low probability of cross-resistance developing between Vip3A and Cry1Ab/c and Vip3A and Cry2Ab as shown in TBW and CEW, and 3) potential to delay development of resistance in other corn varieties expressing Cry toxins. The introduction of MIR162 and its stacks may have an additional benefit of prolonging the lifetime of other corn PIP technologies by providing another mode of action for ECB, CEW, FAW, and WCRW. Generally, the greater the modes of action (i.e. toxin mosaic) in the landscape, the less likely resistance will develop to any one toxin.

Recommendations

BPPD requests that Syngenta explain their future efficacy results more thoroughly especially with respect to significant differences between treatments, what those differences could mean, and what effects might be their underlying causes. Furthermore, Syngenta should provide an explanation in the methodology section of their efficacy studies that supports the use of very small sample sizes (i.e. what assumptions were made and what was the statistical power).

A. BACKGROUND

Syngenta's Bt11xMIR162xMIR604 is a stacked transgenic corn trait that expresses the two registered crystal protein toxins Cry1Ab and mCry3A and incorporates the novel Vip3Aa20 Bt toxin (99.9% identical in amino acid sequence to the Vip3Aa19 produced in COT102). The Vip3A is different from Cry proteins as it is produced during vegetative growth of the bacteria, does not form parasporal crystal proteins, and is secreted (but not processed upon secretion) from the cell as a soluble protein. While its physical manifestations of intoxication resemble those of Cry proteins (gut paralysis and lysis of midgut epithelial cells) (Schnepf *et al.* 1998), activated Vip3A does not bind to the same receptors (APN and cadherin-like receptor). These two types of Bt proteins (Vip, Cry) do not appear to share binding sites.

Syngenta received an Experimental Use Permit (EUP) to allow field testing of PIP Corn Event MIR162 and its combined trait hybrids, MIR162xBt11 and Bt11xMIR162xMIR604, in 23 states to cover the period from March1, 2007 through February 29, 2008. Event MIR162 corn expresses the Vip3A insect control protein. The variant protein Vip3Aa20 produced in MIR162 has insecticidal activity against several lepidopteran pests of corn and specifically targets two major corn pests *Helicoverpa zea* (corn earworm, CEW) and *Spodoptera frugiperda* (fall armyworm, FAW) but is also effective against *Diatraea grandiosella* (southwestern cornborer, SWCB). Vip3A does not have insecticidal activity against *Ostrinia nubilalis* (European cornborer, ECB). The toxin Cry1Ab expressed in Bt11 field corn is highly selective and effective against ECB and SWCB. In addition, Bt11 is also effective against CEW and FAW. The modified toxin mCry3A, as expressed in MIR604, has insecticidal activity against two major coleopteran pests of corn, *Diabrotica longicornis barberi* Smith and Lawrence (northern corn rootworm, NCRW) and *Diabrotica virgifera virgifera* (western corn rootworm, WCRW) but no activity against lepidopteran target pests.

The Bt11 maize (Cry1Ab) benefits have been previously discussed in the 2001 *Bt* crop reassessment document and can be viewed online at http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm.

Benefits resulting from the introduction of MIR604 maize (modified Cry3A) have been published in the Biopesticide Registration Action Document for mCry3A (2007) and can be

viewed online at
http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006509.pdf.

BPPD (2007) reviewed the results of a small scale field trial conducted at multiple locations during the 2005 corn growing season. The review concluded that MIR162 provides significant crop protection against feeding damage caused by *Agrotis ipsilon* (black cutworm, BCW), FAW, CEW and *Striacosta albicosta* (western bean cutworm, WCBW). The level of protection provided by MIR162 is significantly better than that provided by Bt11 alone or a negative isoline with a conventional insecticide standard. Small scale field tests demonstrated that Bt11xMIR162 stacked hybrids controlled BCW, FAW, CEW, WCBW, and ECB. When MIR604 was combined with MIR162, there was some evidence of a possible synergistic effect in the control of corn rootworm in 2005.

In this review, BPPD will discuss and present benefits resulting from the introduction of the stacked Bt trait product Bt11xMIR162xMIR604 and its conclusion of Syngenta's efficacy studies for ECB, CEW, FAW, and WCRW. Insecticidal efficacy of the two combined events Bt11xMIR162 against the major lepidopteran pests (ECB, CEW, and FAW) will also be discussed and compared to the efficacy of Bt11xMIR162xMIR604 maize to look for possible synergistic effects.

B. SYNGENTA'S EXECUTIVE SUMMARY OF IT'S PUBLIC INTEREST DOCUMENT

"Syngenta is seeking registration for a new plant-incorporated protectant, the Vip3Aa20 derived from *Bacillus thuringiensis* as produced in maize transformation event MIR162. Syngenta is also seeking registration for two combined trait maize cultivars containing MIR162 and two other registered plant-incorporated protectants, Cry1Ab in Bt11 maize and mCry3A in MIR604 maize. The first combined trait product will be a breeding cross of MIR162 and Bt11, designated Bt11xMIR162, and the second will be a breeding cross of MIR162, Bt11, and MIR604, designated Bt11xMIR162xMIR604. Data has been developed by Syngenta demonstrating that issuance of these registrations will be in the public interest."

"Field efficacy trials demonstrate that MIR162 maize and Bt11 x MIRI62 maize hybrids provide improved protection against lepidopteran insect feeding damage when compared to the protection provided by conventional insecticides or Bt11 maize alone. This improved product efficacy is expected to translate into increased maize grain yield and quality. In a time of rising demand for maize grain, the MIR162 trait has the potential to provide U.S. agriculture with an economic benefit exceeding $371 million annually at product maturity. The introduction of the MIR162 trait in combination with Bt11 also has the potential to replace many conventional insecticide applications, reduce greenhouse gas emissions, and mycotoxin contamination of livestock feed. There will also be IRM benefits stemming from the introduction of these combined trait hybrids. The Vip3Aa20 protein contained in MIR162 maize brings a second mode of action against *Helicoverpa zea* and *Spodoptera frugiperda*, two pests that are only suppressed by CrylAb. Data has been developed showing that Bt11 x MIR162 is high dose against these two pests; accordingly a reduction from the 50% structured refuge requirement in the South is warranted. This will greatly benefit maize growers in the affected counties of the South as it will allow them to protect more of their maize acres against feeding damage from lepidopteran pests. Adoption of Bt11xMIR162xMIR604 hybrids by growers is predicted to offer crop yield advantages and important new options for control of virtually all the major insect pests of maize, all built into a single seed product. The availability of a new product for lepidopteran and rootworm control will provide choices for growers in the marketplace, and lead to increased price competition for traits, which will benefit growers and others in the maize value chain. Bt11 x MIR162 x MIR604 maize also offers health and environmental safety advantages over the use of conventional insecticides, as well as insect resistance management benefits that will preserve the durability of this and other *Bt*-based products."

"Collectively, the information presented in this document convincingly supports a public interest finding for registration of the plant-incorporated protectants in MIR162, Bt11xMIR162, and Bt11xMIR162xMIR604 maize."

C. BPPD's REVIEW OF SYNGENTA'S PID

Syngenta submitted several documents which will be summarized, reviewed, and analyzed here: efficacy studies conducted during 2006 (White et al. 2007, MRIDs 470530-01, 470530-02, 471530-03, 471530-04, 470531-01, 470531-02, and 470531-03), a Public Interest Document (Ward & Vlachos, 2007, MRID 471378-19), and an IRM chapter (Kurtz et al. 2007, MRID 471374-07) will be addressed in this review as is applicable. A complete IRM review will be done separately.

1. Public Interest Finding

a) *Syngenta's Public Interest Findings* (MRID 471378-19):

BPPD summarized the public interest findings from the PID here. The Human Health and Environmental Benefits as well as Resistance Management benefits reported by the registrant are summarized by BPPD in section 4 and 5 of this document, respectively.

i. Presumption of public interest

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

ii. Need factors

"As the price of maize grain continues to rise, the economic threshold for growers to respond to infestations of *A. ipsilon*, *H zea*, *S. albicosta*, or *S. frugiperda* will fall. Even relatively small reductions in crop yield (< 10%) will result in a significant economic loss for growers. Additionally, there is evidence that populations of *S. albicosta* are spreading eastward and will have the potential to cause greater harm in critical maize producing states. Control of aboveground maize insect pests is challenging for growers. Conventional insecticide applications are costly and intensive scouting of fields is required to identify the appropriate timing for applications. Growers only have a very narrow time window during which insecticides can be applied because many of the above- ground feeding insects are shielded from contact with the insecticides by virtue of their feeding location on the plant. Planting of combined-trait hybrids containing MIR162 will provide growers with a more effective means of controlling these economically significant insect pests of maize."

"While it has not been possible to conduct direct side-by-side efficacy comparisons of CrylF and Bt11xMIRl62 hybrids, Bt11xMIR162 hybrids are expected to provide a level of broad lepidopteran control that is unsurpassed by currently available Bt hybrids or conventional insecticide products. For *H. zea*, in particular, Bt11xMIR162 hybrids have been shown to provide excellent control that meets EPA insect resistance management criteria for 'high dose', whereas CrylF hybrids provide only 'suppression' of this pest."

"Direct efficacy comparisons of Bt11xMIRl62xMIR604 hybrids with other stacked transgenic maize hybrids offering combined lepidopteran and coleopteran control have not been possible. It is expected that Bt11xMIR162xMIR604 hybrids will provide unsurpassed control of target pests. Their excellent broad-lepidopteran control, particularly for *H. zea* and *S. albicosta*, can potentially result in better performance than competitor offerings."

iii. Composition factor

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

iv. Usage factor

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

v. Performance factor

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

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

"Bt11xMIR162 maize will combine the efficacy of Bt11 maize and MIR162 maize to provide broad-spectrum control of major U.S. lepidopteran maize pests at a level that will outperform current technologies. Collectively, the results of field efficacy trials demonstrate that Bt11xMIR162 maize will be protected from feeding damage caused by the following insect pests: *O. nubilalis*, *D. grandiosella*, *D. crambidoides*, *H. zea*, *S. frugiperda*, *P. nebris*, *D. saccharalis*, *A. ipsilon*, *S. albicosta*, and *S. exigua*."

"Combining CrylAb, Vip3Aa20, and mCry3A traits in a single maize hybrid retains the insect control efficacy of the individual proteins. Accompanying the present submission are reports of efficacy studies in *O. nubilalis*, *H. zea*, *S. frugiperda,* and *D. virgifera virgifera* that substantiate the predicted efficacy of combining multiple insecticidal traits in Bt11xMIR162xMIR604 maize hybrids. Therefore, it is reasonable to assume that growers will realize the cumulative benefits of all three insecticidal traits in this product."

vi. Risk factors

"*Fusarium* ear rot is the most common ear rot disease in the Midwest and is closely associated with insect feeding damage to maize ears. Although the disease does not cause significant yield loss, it reduces grain quality, and increases the fungi that can produce mycotoxins, such as fumonisins. Mycotoxin contamination of maize grain presents a potential threat to livestock health and it is occasionally necessary to reject or reformulate field lots because of contamination. Due to the superior protection from insect ear feeding damage that will be afforded by planting MIR162 hybrids there is a potential health benefit for the livestock industry resulting from reduced mycotoxin levels in livestock feed. Thus, the introduction of MIR162 technology has the potential to reduce applications of conventional insecticides and improve grain quality by reducing mycotoxin levels. These facts indicate that registration of MIR162 is in the public interest."

"An additional food and feed safety benefit of Bt11xMIR162 is its potential to reduce the level of fumonisin, a harmful fungal toxin, in maize grain. Grain from Bt maize hybrids (including Bt11 maize) is associated with significantly reduced levels of fumonisin. This is an indirect benefit of protecting maize ears from feeding damage by lepidopteran pests. The additional control of ear-feeding pests that will be provided by Bt11xMIR162 maize particularly for *H. zea* and *S. albicosta* will likely further reduce mycotoxin contamination in grain."

b) *BPPD's response*

Syngenta's claims that "Bt11xMIR162xMIR604 is expected to provide unsurpassed control of target pests" and that the products "broad-lepidopteran control can potentially result in better performance than competitor offerings". BPPD notes that these statements are unverified assumptions.

BPPD concludes that MIR162, Bt11xMIR162, and Bt11xMIR162xMIR604 maize are expected to provide the public interest benefits shared by other corn PIPs already registered by the Agency. Specifically the stacked product would provide a new tool for farmers who face challenges of protecting corn crops from lepidopteran as well as coleopteran pest damage. In addition, the stacked product can be expected to prolong the life-time of other corn PIPs due to Vip3A having a novel mode of action.

A more detailed analysis of benefits derived from human health and environment and IRM will follow in sections 4 and 5.

2. Efficacy Data

"Bt11 maize plants express a truncated Cry1Ab protein for control of certain lepidopteran pests (i.e. ECB) and a phosphinothricin acetyltransferase (PAT) protein that confers tolerance to herbicide products containing glufosinate. MIR162 maize plants express Vip3Aa20 protein to control of FAW, CEW, and WBCW (and other lepidopteran pests) and a phosphomannose isomerase (PMI) protein that acts as a selectable marker trait enabling transformed plant cells to utilize mannose as a primary carbon source. MIR604 maize plants express modified Cry3Aa protein for control of certain coleopteran pests (i.e. WCRW, NCRW) and a similar PMI protein as a selectable marker. GA21 maize plants express a double-mutated 5-enol pyruvylshikimate-3-phosphate synthase (mEPSPS) protein that confers tolerance to herbicide products containing glyphosate."

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

a) <u>Efficacy of Bt11xMIR162xMIR604xGA21 against ECB</u> (MRIDs 471530-01):

The objective of the study was to test whether ECB control efficacy by Bt11 plants is unaffected by the presence MIR162, MIR604, and GA21 or absence of these transgenic traits. The experiment was conducted as a randomized complete block design with three replicates (10 plants each; n=30) in Minnesota and Illinois. The four treatments were Bt11xMIR162xMIR604xGA21, MIR162, Bt11, and a non-transgenic hybrid. Two artificial infestations (simulating 2 generations of ECB in the field) were conducted with laboratory reared neonates at a rate of 150 larvae per plant during the first application (at whorl stage) and 200 larvae per plant during the second application (at pollen shed stage). Foliar leaf damage was assessed using the Guthrie scale of 1-9 (See Appendix A for details) for ten consecutive plants in a row 14 days after first infestation. Forty-five days after the second infestation, ten consecutive plants were dissected to assess ear shank, ear kernel, and stalk feeding by measuring feeding tunnel lengths. Both types of data collected were analyzed using ANOVA.

No significant difference ($p \leq 0.05$) in ECB efficacy (foliar leaf damage; ear shank and stalk feeding damage) was observed between Bt11 and Bt11xMIR162xMIR604xGA21 plants when the data from both locations were pooled or analyzed separately. There was one occasion of significant difference in second generation ECB ear feeding damage at the Illinois location. However, this significant difference disappeared when data were pooled. Syngenta did not provide an explanation as to why there might have been a significant difference at the Illinois location. Significant differences were observed between Bt11 containing hybrid plants and MIR604 and control plants when data were separated by location or pooled (foliar leaf damage; ear, ear shank, and stalk feeding damage). Bt11 containing hybrid plants provided excellent protection against ECB; damage to MIR604 and control plants was much higher.

b) Efficacy of Bt11xMIR162xMIR604xGA21 against CEW (MRIDs 471530-02):

The objective of study was to test the hypothesis that CEW control efficacy by MIR162 plants is unaffected by the presence Bt11, MIR604, and GA21 or absence of these transgenic traits. The experiment was conducted as a randomized complete block design with three replicates in Iowa (five plants/replicate) and Illinois (six plants per replicate; n=18). Artificial infestations were conducted with laboratory reared neonates; approximately 20 larvae per plant were applied to green silks of the most developed ear on each plant. Ear feeding damage was assessed using the modified Widstrom scale (See Appendix B for details) 14 days after infestation. Data collected were analyzed using ANOVA.

There was a significant difference in ear feeding damage between Bt11xMIR162xGA21 plants and MIR162 plants as compared to Bt11 plants indicating that MIR162 provides excellent protection against CEW damage. While there was no statistically significant difference between the two hybrids containing MIR162, ear feeding damage was numerically higher on Bt11 x MIR162 plants than on MIR162 plants alone, suggesting that Bt11 provides some protection against CEW. Damage on control plants was statistically significant from damage caused on Bt11 plants supporting that Bt11 provides some protection from CEW damage.

c) Efficacy of Bt11xMIR162xMIR604xGA21 against FAW (MRIDs 471530-03):

The objective of the study was to test that FAW control efficacy by MIR162 plants is unaffected by the presence of Bt11, MIR604, and GA21 or absence of these transgenic traits. The experiment was conducted as a randomized complete block design with three replicates (10 plants each; n=30) in Minnesota and Illinois. Two artificial infestations were conducted with lab reared neonates; approximately, 80 larvae per plant were placed into the whorl of each plant. Foliar feeding damage was assessed 14 days after infestation using a modified version of the Davis scale (See Appendix C for details). Data collected were analyzed using ANOVA.

Plants containing either MIR162 alone or MIR162 with other traits suffered slight damage from FAW larvae; damage ratings differed significantly from Bt11 ratings. FAW were destructive to non-transgenic corn plants, and those damage ratings differed significantly from all other treatments. The results confirm that plants containing the MIR162 insecticidal trait alone or in a stack as Bt11xMIR162xMIR604 provide excellent control against FAW.

d) Efficacy of Bt11xMIR162xMIR604xGA21 against WCRW (MRIDs 471530-04):

The objective of the study was to test that CRW control efficacy by MIR604 plants is unaffected by the presence of Bt11, MIR162, and GA21 or absence of these transgenic traits. The experiment was conducted as a randomized complete block design with three replicates (6 plants each; n=18) in Minnesota and Illinois. The three treatments were Bt11xMIR162xMIR604xGA21, MIR604, and a non-transgenic hybrid. One artificial infestation was conducted with WCRW eggs at a rate of 1500 eggs per plant at V2-V3 stage of plant development. In Illinois, the trial was conducted in a field which had been planted to a trap crop for WCRW the previous season to attract beetles for increased egg accumulation. Damage ratings (see Appendix D for details) were taken on roots collected and washed just prior to the silk stage. Six root samples per plot were selected at both locations. ANOVA was used to analyze the data.

Plants containing MIR604 had significantly less damage than control plants. No statistical difference was detected between MIR604 plants and Bt11xMIR162xMIR604xGA21 plants.

e) Efficacy of Bt11xMIR162xGA21 against ECB (MRID 471531-01):

The objective of study was to compare the ECB control efficacy by Bt11xMIR162xGA21 plants to the efficacy of hybrids containing only MIR162 or Bt11. Study methodology was identical to ECB study mentioned under section a).

No significant difference ($p \leq 0.05$) in ECB efficacy (foliar leaf damage) was observed between Bt11 and Bt11xMIR162xGA21 when the data from both locations were analyzed separately or pooled. Both hybrids provided excellent protection against first generation ECB. Significant differences were observed between Bt11 containing hybrid plants and MIR604 and control plants when data were separated by location or pooled. Second generation ECB damage to MIR162 and control plants was significantly higher than to Bt11xMIR162xMIR604 plants. Both hybrids containing Bt11 provided excellent protection against ECB. There was a small yet statistically significant level of suppression at one location (IL) by MIR162 plants against ECB ear and stalk feeding when compared to control plants.

f) Efficacy of Bt11xMIR162xGA21 against CEW (MRID 471531-02):

The objective of study was to compare the CEW control efficacy by Bt11xMIR162xGA21 plants to the efficacy of hybrids containing only MIR162 or Bt11. Study methodology was identical to ECB study mentioned under section b).

CEW larvae cause slight damage to treatment plants containing the MIR162 event and there was no statistical difference between damage ratings of the two treatments MIR162 and Bt11 x MIR162xMIR604xGA21. There was a significant difference in ear feeding damage between the two treatments containing MIR162 alone or in the stack and the Bt11 treatment (IL location). When the data were pooled from both locations, the difference disappeared. Syngenta did not provide an explanation for this result. However, BPPD noticed that mean ear rating reported for the Bt11 treatment was much greater than that reported for the IA location. Damage on control plants was statistically significant from damage caused on Bt11 plants supporting that Bt11 provides some protection from CEW damage. Damage to MIR162 containing hybrids was slight.

g) Efficacy of Bt11xMIR162xGA21 against FAW (MRID 471531-03):

The objective of the study was to test that FAW control efficacy by MIR162 plants is unaffected by the presence or absence of Bt11. Study methodology was identical to ECB study mentioned under section c).

Both treatment plants containing MIR162 alone or stacked with Bt11xGA21 provided excellent control against FAW, and no significant difference between the two damage ratings was observed. The Bt11 treatment plants suffered greater damage and their damage rating differed significantly from the two MIR162 treatment ratings. The control plants suffered the greatest damage, and their damage rating differed from all other treatments.

BPPD's response

BPPD (2007) reviewed the results of a small scale field trial conducted at multiple locations during the 2005 corn growing season. One of BPPD's comments in that review was that MIR604, when combined with MIR162, showed some evidence of a possible synergistic effect in the control of corn rootworm. In Syngenta's newest efficacy studies reviewed above (2006 growing season), the samples sizes chosen per treatment are extremely small (N_{WCRW}=6, 5 and N_{WCRW} = 18 and 15). The power to detect significant differences between treatments of i.e. WCRW depends heavily on the sample size chosen. BPPD is concerned that Syngenta may not have a basis for testing there hypothesis that "the efficacy of MIR604 in plants is unaffected by the presence or absence or other *Bt* traits" or whether there are synergistic effects when MIR604 is combined with MIR162. Specifically, BPPD expects the difference in NCRW damage to be smaller between MIR604 and Bt11xMIR162xMIR604xGA21 plants than between MIR604 and control plants and has doubts that Syngenta's experiments do have enough power to detect these smaller differences between treatments due to the very small sample size chosen. BPPD would like to know what the rational was for choosing such small samples.

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

- There are indications from the Illinois data (ECB and CEW efficacy studies) that stacking the traits Bt11, MIR162, MIR604, and GA21 maize together may produce different efficacy results than for Bt11 plants alone. Whether this is due to synergistic effects between the toxins or environmental effects is unclear, and the registrant did not provide an explanation for the results. In future submissions of efficacy studies, BPPD requests that Syngenta address such differences by supplying possible explanations. However, BPPD notes that both the stacked and single trait containing products appear to provide good protection against ECB and CEW.

- The data support that the stack containing Bt11, MIR162, MIR604 and GA21 traits provides good efficacy results against FAW.

- The data support that the stack containing Bt11, MIR162, MIR604 and GA21 traits produces reasonably good efficacy against WCRW.

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

3. Grower Benefits

a) *Syngenta's Review and BPPD's summary of reports in the PID:*

Economic Benefits

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

> "The introduction of a new technology will have an effect on the market for existing technologies that is beneficial to users of either technology. This will come in the form of downward pressure on prices of the competing technologies. This is beneficial to growers because prices of maize traits will tend to remain lower and more stable in the future than would otherwise be the case."
>
> "From data collected in a telephone survey of 150 maize growers in 12 states, average yield losses in 2006 attributable to *H. zea* were estimated to be 4.9 bu/ac and losses attributable to *S. albicosta* were estimated to be 4.8 bu/ac. Examination of data provided by these growers for the past five seasons suggests that yield losses attributable to the two pests are increasing. This conclusion is supported by analysis of insecticide use data for 2005 and 2006, which indicate that economically significant infestations of *H. zea* and *S. albicosta* are on the rise in the Corn Belt and Great Plains. As the price of maize grain increases, the amount of feeding damage needed to exceed an economic threshold for applying corrective measures decreases. The grower survey results indicate that 70% of respondents would purchase MIR162 hybrids if they were available and would plant them on an average of 500 acres per farm four years after introduction."
>
> "A potential economic benefit for maize growers from the commercial introduction of MIR162 hybrids has been computed in the form of an upper-bound estimate for the three largest maize producing states (IA, IL, and NE). Effective control of *H. zea* and *S. albicosta* in these three states alone provides an economic benefit of up to $371 million annually assuming a 100% market share for MIR162 hybrids (not taking into account potential price responses for competitive products). These substantial economic benefits indicate that registration of MIR162 is in the public interest."
>
> "For many growers, the broad lepidopteran control offered by Bt11xMIR162 hybrids will represent a higher insurance value than currently available *Bt* products. The need to scout fields for pest pressure or to apply other control measures for lepidopteran larvae will be reduced."
>
> "The broad efficacy of Bt11xMIR162xMIR604 hybrids will provide 'insurance' for growers against damage by multiple pests that might otherwise cause significant economic loss in any given year. The same broad efficacy will provide convenience for

growers, as they will be able to eliminate the need to apply both a soil insecticide for control of *Diabrotica* rootworms and *A. ipsilon*, and a foliar insecticide later in the season for foliar insects. It will also reduce or eliminate their need to scout fields for pest pressure."

In addition, commodity prices for maize grain have dramatically increased recently due to high demand for fuel ethanol production, and sustained demand is predicted for the coming years. Such demand will function to increase the value of a grower's investment in any agricultural practice, technology, or product, including the Bt11xMIR162 hybrids and Bt11xMIR162xMIR604 traits that increases or preserves yield. Another predicted economic benefit is increased competition in the marketplace for pest-control products. The introduction of Bt11xMIR162xMIR604 hybrid maize seed will increase grower choice and can be expected to exert downward pressure on the cost of all products that offer control of lepidopteran pests in the case of Bt11xMIR162 and rootworm pests in the case of Bt11xMIR162xMIR604 hybrids.

b) *BPPD's response*

BPPD focuses on the benefits from MIR162, Bt11xMIR162, and Bt11xMIR162xMIR604 only and not on the potential economic benefits of these products containing the GA21 trait. It may be that additional benefits are derived from an herbicide tolerant trait in MIR162, Bt11xMIR162, and Bt11xMIR162xMIR604. On the other hand, such a trait could also increase the risk of weed resistance.

BPPD finds that MIR162, Bt11xMIR162, and Bt11xMIR162xMIR604 will likely have similar general economic grower's benefits of already registered corn PIPs (Bt11 and MIR604) as described by the Agency in the 2001 *Bt* crop reassessment and MIR604 BRAD. The Agency's summary of these benefits can be accessed online at http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm and (http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006509.pdf). In addition, Bt11xMIR162xMIR604 maize will provide further benefits by controlling corn root worm as well as several lepidopteran pests.

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

4. Human Health and Environmental Benefits

a) *Syngenta's Review* (MRID 471378-19):

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

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

"The combined mammalian and environmental safety profile of Bt11xMIRl62 indicates that the product will pose no significant risks. Accordingly, it offers health and environmental advantages over current chemical alternatives for control of lepidopteran pests."

"The combined mammalian and environmental safety profile of Bt11xMIR162xMIR604 maize indicates that the product will pose no significant safety risks. Accordingly, it offers significant health and environmental advantages over current chemical alternatives for control of lepidopteran and rootworm pests. For maize growers who currently rely upon conventional insecticide applications for lepidopteran and rootworm control, Bt11xMIR162xMIR604 maize will allow them to significantly reduce, if not eliminate, the need to apply chemical controls for these pests. This will represent both a reduced health and safety risk for agricultural workers and will reduce the impact of insecticide use on wildlife and the environment."

b) *BPPD's response*

EPA's review of Syngenta's human health and environmental data is pending. Syngenta claims that there is no reason for concern with respect to hazard and allergenicity for the proteins expressed in MIR162, Bt11xMIR162, and Bt11xMIR162xMIR604. From the knowledge of other PIPs, BPPD expects the results for human health and environment to be similar to already registered PIPs and not to pose a problem. However, BPPD will confirm this upon finalizing its review of Syngenta's submitted data. For information regarding the Agency's conclusion on Bt11 benefits with respect to human health and environment, the 2001 *Bt* crop reassessment (http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad.htm) and the Biopesticides Registration Action Document for the modified Cry3A protein (http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006509.pdf) can be consulted.

5. Resistance Management Benefits

a) *Syngenta's Review and BPPD Summary of Benefits of IRM Submission* (MRIDs 471378-19 and 471374-07):

"Use of Bt11xMIRl62 maize offers insect resistance management benefits that will help to preserve the durability of this and other Bt-based products for lepidopteran control. The CrylAb and Vip3Aa20 proteins are present in these hybrids at levels that have been demonstrated to provide a high-dose for control of *O. nubilalis*, *H. zea*, and *S. frugiperda*, thus minimizing the risk of resistance developing in these species. Bt11xMIR162 hybrids offer IRM advantages in comparison to other control options that do not demonstrably provide a 'high dose' against the target pests. Moreover, Vip3Aa20 operates by a mode of action different from that of Cryl Ab or Cryl F and targets a unique binding site(s) in susceptible larvae. The available data support a conclusion that Vip3Aa20 shows no potential for cross-resistance with Cry proteins. Thus, for *H. zea* and *S. frugiperda*, which are sensitive to both CrylAb and Vip3Aa20, Bt11xMIR162 maize is predicted to significantly extend the durability of both traits for control of these pests because local populations are very unlikely to evolve resistance to two proteins that act on independent target sites."

"The possibility of resistance development in *H. zea* has been of particular concern to the EPA, as it is also a pest of cotton and has the potential to undergo selection pressure from both *Bt* maize and Bt cotton varieties that express similar Cry proteins where the two crops are grown in the same geographies. The principal reason that the EPA requires growers in cotton-growing areas to plant 50% of their maize acres to non-Bt maize hybrids concerns the potential for resistance evolution in *H. zea* populations. Syngenta provides data and rationale to justify reduction of the maize refuge in cotton-growing areas from 50% to 20% of maize acres for growers of Bt11xMIR162 maize. No other *Bt* product offers comparable IRM advantages in maize. "

"For growers of Bt11xMIR162 maize hybrids, the reduced refuge requirement in cotton-growing regions will translate into a higher proportion of insect-protected maize acres with a proportional increase in all the attendant benefits of the product in these areas. As an added advantage, compliance with the refuge requirement for IRM can be predicted to increase because *Bt* maize growers in cotton-growing regions have heretofore not been able to fully experience the benefits enjoyed by *Bt* maize growers in other regions of the U.S. The potential for increased maize acres in cotton-growing regions can also help meet the current high demand for maize grain."

"The same insect resistance management benefits for Bt11xMIR162 maize will also apply to Bt11xMIR162xMIR604 maize. Accordingly, a 20% non-*Bt* maize refuge in cotton-growing regions will be justified. The stacking of three insecticidal proteins in this product is not expected to increase selection pressure for cross-resistance among local pest populations, owing to the different modes-of-action and target sites for the CrylAb, Vip3Aa20, and mCry3A proteins. Because the mCry3A trait in Bt11xMIR162xMIR604 maize has good efficacy against its target rootworm pests, introduction of this product is expected to help extend the durability of other commercially available rootworm-protected *Bt* maize products."

b) *BPPD's response*

BPPD will respond to the refuge reduction request separately in the IRM review for Bt11xMIR61xMIR604. However, should the refuge be reduced, growers in the affected areas will likely realize some economic benefits.

The BPPD IRM team concluded the following based on the "dose" data submitted by the registrant (MRID 471374-07):

- Bt11 expresses a "non high-dose" and has low activity against FAW, "non high-dose" against CEW, "near high-dose" and high-dose against ECB under verification methods #1 and #4.

- MIR162 expresses a high-dose against FAW, non high-dose and "near high-dose" against CEW, and no activity against ECB under verification methods #1 and #4.

- Bt11xMIR162 expresses a high-dose against FAW, "near high-dose" and "effective high-dose" against CEW, and near high-dose and high-dose against ECB under verification methods #1 and #4.

Syngenta commission Dr. Caprio to evaluate and model the following scenarios for CEW:

1) 20% sprayed cotton non-Bt refuge with 80% VipCot™, and 50% sprayed corn non-Bt refuge with 50% Bt11xMIR162; and
2) 20% sprayed cotton non-Bt refuge with 80% VipCot™ and 20% sprayed corn non-Bt refuge with 80% Bt11xMIR162; and
3) A series of single gene Bt (Cry 1Ab/c) cotton and corn planted along with Vip3A cotton and corn stacks

Dr. Caprio concluded from his simulations that when refuge was reduced to 20% in the cotton growing region and no single-gene crop was present, resistance did not evolve to either Cry1Ab or Vip3A. The simulations further suggest that within 25 years, there is little risk of CEW resistance evolving to the Bt11xMIR162 stack whether 50% or 20% non-Bt refuge is planted in cotton growing regions. In 80% of the simulations, resistance evolved to Cry1Ab during a 25 year period when a single gene crop was planted. The more single gene crop was planted, the faster resistance evolved to Cry1Ab/c. When no single-gene crop was present, resistance did not evolve to either Cry1Ab or Vip3A.

BPPD notes that the dose assumptions for VipCot™ and MIR162 with respect to CEW (based on lab bioassays and field studies) appear realistic so that those model input parameters can be relied upon with a good degree of confidence. BPPD concludes that MIR162, Bt11xMIR162, and Bt11xMIR162xMIR604 have the following benefits: 1) high dose against FAW, ≥ near high-dose against CEW, and ≥ near high-dose against ECB, 2) low probability of cross-resistance developing between Vip3A and Cry1Ab/c and Vip3A and Cry2Ab as shown in TBW and CEW, and 3) potential to delay development of resistance in other corn varieties expressing Cry toxins. The introduction of MIR162 and its stacks may have an additional benefit of prolonging the lifetime of other corn PIP technologies by providing another mode of action for ECB, CEW, FAW, and WCRW. Generally, the greater the modes of action (i.e. toxin mosaic) in the landscape, the less likely resistance will develop to any one toxin.

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Appendix A. Leaf Damage Rating Scale for ECB (Guthrie et al. 1960)

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

Appendix B. Ear Feeding Damage Rating for CEW (Widstrom 1967)

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

Appendix C. Modified Davis Scale for Foliar Feeding Damage Ratings by FAW (Davis et al. 1992)

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

Appendix D. Linear Root Feeding Rating Scale for WCRW (adapted from Oleson 2005)

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