## Response to Public Comments on Syngenta SYN-05307-1 Corn:

On July 13, 2012, APHIS published a notice in the Federal Register (77 FR 41366-41367, Docket no. APHIS-2012-0024) announcing the availability of the Syngenta petition, and the APHIS PPRA and draft EA for a 60-day public review and comment period. Comments were required to be received on or before September 11, 2012.

APHIS received a total of 86 comments from various individual and groups on the SYN-05307-1 corn petition (hereafter referred to as 5307 Corn), PPRA, and draft EA. The majority of the comments opposed the development of genetically engineered foods and/or 5307 Corn. Public comments included individual submissions and form letters encompassing both the peer-reviewed and non-peer-reviewed literature. Fourteen public comments supporting a determination of nonregulated status of 5307 Corn were submitted from corn grower associations, agribusiness associations, and a state Farm Bureau. Those individuals cited several salient points regarding the potential benefits of 5307 Corn, including that 5307 Corn will help manage corn rootworm resistance and provide significant economic savings to U.S. growers.

Those 41 public comments received opposing an approval of Syngenta's request for nonregulated status for 5307 Corn were submitted by individuals and a Non-Government Organization (NGO). One of the comments was a letter with 4,601 identical letters attached to it. Nineteen of the public comments contained only references, with no other information. Many of the public comments expressed a general opposition to genetically modified organisms (GMOs) or GE crops and the domestic regulatory process surrounding GE plants; perceived negative effects on public and animal health, biodiversity, and the environment; and a lack of consideration regarding organic production systems and the public right to choose non-GE containing food products. The majority of these public comments did not explain or identify elements in the 5307 Corn PPRA or EA that were perceived to be inadequate or provide any supporting evidence for their claims. Several specific issues related to the 5307 Corn EA were, however, identified from the collective pool of public comments and form letter submissions. These were organized into categories and addressed below.

**Comment 1: One commenter stated that because 12 dockets for petitions were posted on the same day, that the public was not afforded enough time to review the documents.**

Most of the other dockets available for review were entered into the improved process; the public had an initial opportunity to assess issues associated with the petitions, and respond. A thorough regulatory review for nonregulated status of these products is yet to be completed. There will be opportunity for the public to comment on the ensuing environmental assessments after they have been published, and if comments about significant impacts have been received, another and final EA will be prepared. Therefore, these other dockets have not necessarily received the final opportunity for public comment. An environmental assessment for this Syngenta 5307 corn and for two other products were available for the 60 day comment period, and APHIS deemed this

sufficient opportunity for the public to provide substantive comments for these three EAs. Following the comment period, the Agency thoroughly reviewed the comments and will have carefully considered other inputs as it prepared APHIS' final plant pest risk assessment, environmental assessment, and possible regulatory determination in response to the petitions for nonregulated status submitted for this and each of the products.

**Comment 2: Four commenters stated that it was necessary for APHIS to conduct a full Environmental Impact Statement (EIS) in order to adequately analyze the issues.**

**APHIS Response:** APHIS recognizes that some citizens are opposed to genetic engineering of food crops. As discussed in the EA, the basic charge of APHIS is to protect American agriculture through improvements in agricultural productivity and competitiveness, and contributions to the national economy and the public health. APHIS asserts that all methods of agricultural production (conventional, organic, or the use of genetically engineered (GE) varieties) can provide benefits to the environment, consumers, and farm income.

Since 1986, the United States government has regulated GE organisms pursuant to a regulatory framework known as the Coordinated Framework for the Regulation of Biotechnology (51 FR 23302, 57 FR 22984) (Chapter 1.6 of the EA). As described in Chapter 1.2 of the EA, APHIS regulates the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products under the authority of the plant pest provisions of the Plant Protection Act and CFR part 340 when APHIS determines that it is unlikely to pose a plant pest risk. Based on scientific information and analysis provided in both the PPRA (USDA-APHIS, 2012) and EA, APHIS has concluded that 5307 Corn does not pose a plant pest risk and will not significantly impact the quality of the human environment, respectively. Due to the lack of significant impacts as presented in the FONSI, an EIS for determination of nonregulated status of 5307 Corn is not necessary.

APHIS relied on a variety of sources to support its analysis of the potential impacts of a determination of nonregulated status of 5307 Corn, including those pertaining to health and the environment. These sources included, but are not limited to, the Syngenta petition, 10-336-01p, and peer-reviewed literature. The analyses in the EA used a variety of expert and technical resources in addition to the 10-336-01p petition. A complete list of references used to support development of the EA can be viewed in the bibliography located in Chapter 8 of the EA.

The EA took a hard look at the need for action, the issues, alternatives, and environmental consequences. APHIS also reviewed the assessment of plant pest risk for Syngenta 5307 corn and carefully considered all comments submitted by respondents to the public involvement efforts. As a result of this analysis, APHIS prepared a final EA, from which came the NEPA decision document and a finding of no significant impact (FONSI) that discussed, under each of the Council of Environmental Quality (CEQ) points of significance, why each point was not

significant, and why an EIS was not required. The agency followed CEQ NEPA regulations and Agency NEPA implementing procedure.

APHIS has determined that the analysis in its EA showed no significant impact on the quality of the human environment if APHIS was to approve a petition for nonregulated status of 5307 Corn..

**Reference**

USDA-APHIS. (2012). Sygenta Company petition for determination of nonregulated status of SYN-05307-1 rootworm resistant corn, draft environmental assessment.

**Comment 3: One commenter stated that plant pests are developing resistance to *Bt* proteins. The commenter cited a 2009 study (Tabashnik et al, 2009a) in which laboratory-maintained and tested populations of the pink bollworm developed cross-resistance between the *Bt* proteins Cry1Ac and Cry2Ab.**

**APHIS response:** The commenter cited a study in which pink bollworm reared and tested in laboratories developed resistance to the Cry1Ac and Cry2Ab *Bt* cotton-derived proteins (Tabashnik et al., 2009a). However, the authors specifically state that this finding does not threaten the efficacy of *Bt* crops in the field because the study was conducted in the lab under artificial conditions not likely to be found in the field. Tabashnik et al. (2009a) note that their findings of lab resistance show the ***potential*** for resistance development and do not demonstrate evidence that resistance occurs in the field with *Bt* crops. Demonstration of resistance to a toxin is dependent on an increased frequency of individual insects which are resistant to a given toxin; detecting the presence of alleles (copies of genes) which confer resistance without also showing that the frequency of individuals containing such alleles within the population are rising does not constitute evidence of field-evolved resistance (Tabashnik et al., 2009b). Despite being exposed to *Bt* toxins, targeted pests remain susceptible to the toxins (Tabasnik et al. 2009a).

An important method of slowing the development of resistance to Bt crops in the field is the use of refuges, areas of field which are planted in a non-Bt crop along with the Bt crop (Bravo et al., 2011). The refuge is intended to maintain a population of insects which are susceptible to the *Bt* toxins. Those insects which are susceptible to *Bt* toxins mate more frequently with individual insects which have the genetic ability to resist the effect of *Bt* toxins (Tabashnik et al., 2008). The refuge strategy assumes that resistant individuals are rare (fewer in number than susceptible insects) (Tabashnik, 2009b) and that these insects will more frequently mate with the susceptible insects found in the nearby refuge. If the ability to resist *Bt* toxins is genetically recessive, then the matings of resistant and the more abundant susceptible insects will produce offspring which are susceptible to *Bt* and will be killed by *Bt* crops. This will also slow the evolution of resistance to *Bt* (Tabashnik et al., 2008). Studies which have monitored resistance of plant pests demonstrate that the refuge strategy of delaying evolution of resistance to *Bt* toxins has been effective (Tabashnik et al. 2008, 2009a).

As analyzed in the EA, although some reports have proposed that resistance to other corn rootworm traits may have been detected, other factors may be responsible for recent incidences of reduced yield caused by corn rootworm in Bt-expressing crops. Also implicated are large rootworm populations exerting pressure on corn that contains only modest dosage levels of the Bt for corn rootworm protection. The Syngenta trait is meant to be stacked with multiple corn rootworm defense genes, including this one, to help deter actual resistance development. See EA sections 2.2.2 and 4.2.2 for a complete analysis.

**References:**


Bravo A, Likitvivatanavong S, Gill S, and Soberon M. (2011). *Bacillus thuringiensis*: a story of a successful bioinsecticide. *Insect Biochem. Molec. Biol*. *41*, 423-431.

Tabashnik B, Gassmann A. Crowder D, and Carriere Y. (2008). Insect resistance to *Bt* crops: evidence versus theory. *Nat. Biotechnol. 26*, 199-202.

Tabashnik B, Unnithan G, Masson L, Crowder D, Li X, and Carriere Y. (2009a). Asymmetrical cross-resistance between *Bacillus thuringiensis* toxins Cry1Ac and Cry2Ab in pink bollworm. *Proc. Nat. Acad. Sci. 106*(29), 11889-11894.

Tabashnik B, Van Rensburg J., and Carriere Y. (2009b). Field-evolved resistance to *Bt* crops: definition, theory, and data. *J. Econ. Entomol*. *102*(6), 2011-2025.


**Comment 4: Several commenters were concerned about potential negative effects of *Bt* crops to nontarget organisms, including arthropods such as butterflies and honey bees, animals such as livestock and humans.**

**APHIS response:** *Bacillus thuringiensis* is a naturally occurring soil bacterium (Lang and Otto, 2010) whose ability to form spores containing insecticidal proteins is one of its cardinal features (Sanahuja et al., 2011). One of the primary reasons for the safety of *Bt* crops as they relate to nontarget organisms such as humans, livestock, and other vertebrates is its species-specificity (Perez-Garcia et al., 2011; Hofmann et al., 2011): that is, *Bt* is only deleterious to insects, and individual Cry proteins used in *Bt* crops only kill certain types of insects (Yu et al., 2011). In particular, Cry1 and Cry2 are toxic for lepidopteran pests, Cry2A for lepidopteran and dipteran pests, and Cry3 for coleopteran pests (Yu et al., 2011). Cry toxins are distinguished and classified according to their primary amino acid sequence (amino acid sequences determine the expression of different proteins) (Bravo et al., 2011). This species specificity is also known as a narrow spectrum of activity (Bravo et al., 2011)

5307 Corn is modified with a Cry3 protein. Activity spectrum data indicate that the insecticidal effects of eCry3.1Ab are limited to certain species of the Chrysomelidae family of Coleoptera.

The eCry3.1Ab protein demonstrates no lepidopteran (insect order which includes butterflies) activity, despite containing sequences from a lepidopteran-active protein (Syngenta, 2011c), which underscores the specificity of the eCry3.1Ab protein

Specificity of the Cry proteins is related to differing receptors in the proteins which affect binding ability to the insect midgut (Then, 2010). When crops are genetically modified to contain *Bt*, feeding by susceptible insects leads to death by the means of disruption of the membranes within the midgut, an organ within the insect digestive system. This membrane disruption leads to a disproportionate influx of water into the midgut, and the insect eventually dies as a result of septicemia and possibly infection by other bacterial species (Abdullah et al., 2009; Bravo et al., 2011).

The species specificity of Bt is also why it is nontoxic to nontarget organisms, including honey bees, livestock, and humans. Duan et al. (2010) reported that exposure to coleopteran-active Cry proteins, such as that found in Cry3, did not significantly reduce lab or field survival of nontarget organisms. An analysis of 42 field experiments indicates that nontarget invertebrates are generally more abundant in *Bt* cotton and Bt maize fields than in nontransgenic field managed with insecticides (Marvier et al., 2007).

**References:**


Abdullah M, Moussa S, Taylor M, and Adang M. (2009). *Manduca sexta* (Lepidoptera: Sphingidae) cadherin fragments function as synergists for Cry1A and Cry1C *Bacillus thuringiensis* toxins against noctuid moths *Helicoverpa zea*, *Agrotis ipsilon*, and *Spodoptera exigua*. *Pest Manage. Sci. 65*, 1097-1103.

Bravo A, Likitvivatanavong S, Gill S, and Soberon M. (2011). *Bacillus thuringiensis*: a story of a successful bioinsecticide. *Insect Biochem. Molec. Biol. 41*, 423-431.

Duan J, Lundgren J, Naranjo S, and Marvier M. (2010). Extrapolating non-target risk of *Bt* crops from laboratory to field. *Biol. Lett. 6*, 74-77.

Hofmann C, Vanderbruggen H, Hofte H, Van Rie J, Jansens S, and Van Mellaert H. (1988). Specificity of *Bacillus thuringiensis* delta-endotoxins is correlated with the presence of high-affinity binding sites in the brush border membrane of target insect midguts. *Proc. Nat. Acad. Sci. 85*, 7844-7848.

Lang A and Otto M. (2010). A synthesis of laboratory and field studies on the effects of transgenic *Bacillus thuringiensis* (*Bt*) maize on non-target Lepidoptera. *Entomol. Exp. et Appl. 135*, 121-134.

Marvier M, McCreedy C, Regetz J, and Kareiva P. (2007). A meta-analysis of effects of *Bt* cotton and maize on nontarget invertebrates. *Science 316*, 1475-1477.

Perez-Garcia A, Romero D, and De Vincente A. (2011). Plant protection and growth stimulation by microorganisms: biotechnological applications of bacilli in agriculture. *Curr. Op. Biotechnol. 22*, 187-193.

Sanahuja G, Banakar R, Twyman R, Capell T, and Christou P. (2011). *Bacillus thuringiensis*: a century of research, development, and commercial applications. *Plant Biotechnol.* J. *9*, 283-300.

Syngenta. (2011). Syngenta Company petition for determination of nonregulated status of SYN-05307-1 rootworm resistant corn. http://www.aphis.usda.gov/biotechnology/not_reg.html)

Then, C. (2010). Risk assessment of toxins derived from *Bacillus thuringiensis*—syngergism, efficacy, and selectivity. *Environ. Sci. Pollut. Res.17*, 791-797.

Yu H-L, Li Y-H, and Wu K-M. (2011). Risk assessment and ecological effects of transgenic *Bacillus thuringiensis* crops on non-target organisms. *J. Integ. Plant Biol. 53*(7), 520-538.

**(a). Three commenters raised the issue of a possible deleterious effect of *Bt* proteins on honey bee learning ability. The commenter referenced a 2008 study by Ramirez-Romero et al., which suggested that high dosages of Bt disturbed learning ability.**

**APHIS response:** Comprehensive reviews of the effects of *Bt* on honeybees have found no detrimental effects (Duan et al., 2008; Yu et al., 2011). Duan et al. (2008) conducted a meta-analysis of data from 25 independent studies of the effects of *Bt* proteins in GE crops to control Coleoptera (beetles) and Lepidoptera (moths and butterflies) and concluded that these proteins do not negatively affect survival of larvae or honeybee adults.

Learning behavior in honeybees is important because foraging worker bees need to be able to appropriately distinguish between flowering plants which contain adequate amounts of nectar and pollen, and those flowering plants in which nectar and pollen are depleted (Seeley, 1985; Hammer and Menzel, 1995). Because the condition of nectar and pollen resources within flowering plants may change very quickly (within a matter of days), bees need to be able to learn and store information related to color and odor of these plants (Behrends and Scheiner, 2009; Srinivasan, 2010). The ability to switch quickly between rewarding and unrewarding plants is critical to foraging honeybees (Herrera, 1990).

The commenter referred to a study in which extremely high doses (5,000 ppb) of the *Bt* toxin Cry1Ab was fed in the form of syrup (a sucrose solution to which Cry1Ab had been added) to young honeybee adults (Ramirez-Romero et al., 2008). Following consumption of *Bt* syrup, the authors asserted that data obtained from a standard behavioral assay, the PER (proboscis

extension reflex) assay (Pham-Delegue et al., 1993), showed disturbances to honeybee olfactory learning behavior. Tests using lower doses (3 ppb) of *Bt*-syrup showed no effect on honeybees.

Subsequent research found results different from Ramirez-Romero et al. (2008). Han et al. (2010) utilized a novel assay consisting of a T-tube maze as well as the PER assay in order to assess learning behavioral abilities of honeybees which had been exposed to Cry proteins from *Bt* cotton pollen. They determined that there were no significant differences between performance of exposed honeybees and control honeybees, and that therefore, the tested Cry proteins did not negatively affect learning in honeybees.

Dai et a.l (2012) tested the effect of *Bt* corn toxins on honeybee performance and learning behavior by placing whole colonies in either *Bt* crop or non-*Bt* crop fields, and comparing the results. They found no significant differences between bees from *Bt* fields or non *Bt* fields in larval stages, body weight, colony performance, foraging activity or learning abilities, and concluded that *Bt* corn has no negative impacts on physiology or learning behavior in honeybees.

Dai et al. (2012) criticized the results from Ramirez-Romero et al (2008) based on a number of factors. They noted that it is often difficult to extrapolate data from tests using purified proteins for feeding, as did Ramirez-Romero et al. (2008), to real-life ecological effects seen in the field. The method of exposure to *Bt* toxins in the purified proteins route may be different from that when using whole plant tissues (such as pollen) to feed and test insects. The use of *Bt*-contaminated syrups by Ramirez-Romero et al. (2008) rather than corn pollen may also be problematical because the *Bt* in the syrup may have resulted in greater bioavailability of the *Bt* toxin and hence, overestimation of the amount of exposure (Dai et al., 2012). Dai et al. (2012) also noted that using laboratory feeding studies to draw conclusions may also be misleading because such lab studies eliminate the social interactions of the honeybee colony, and which therefore have a limited ability to predict the effect of *Bt* crops on honeybee colonies under conditions seen in agricultural fields.

Similarly, Cry3B proteins have no toxicity to bumblebees (Duan et al, 2008), and recent results have been obtained when testing the effect of *Bt* on genetically related species of bumblebees. Arpaia et al. (2012) found that a Cry3Bb1-expressing tomato line does not negatively affect feeding behavior of foraging bumblebees.

**References:**


Arpaia S, Battafarano R, Chen L-Y, Devos Y, Di Leo G, and Lu B-R. (2012). Assessment of transgene flow in tomato and potential effects of genetically modified tomato expressing Cry3Bb1 toxins on bumblebee feeding behavior. *Annals Appl. Biol. 161,* 151-160.

Behrends A and Scheiner R. (2009). Evidence for associative learning in newly emerged honey bees (*Apis mellifera* L.). *Anim. Cogn. 12,* 249-255.

Dai P-L, Zhou W, Zhang J, Cui H-J, Wang Q, Jiang W-Y, Sun J-H, Wu Y-Y, and Zhou T. (2012). Field assessment of *Bt* Cry1Ah corn pollen on the survival, development, and behavior of *Apis mellifera ligustica*. *Ecotoxicol. Environ. Safety 79*, 232-237.

Duan J, Marvier M, Huesing J, Dively, G, and Huang G. (2008). A meta-analysis of effects of *Bt* crops on honey bees (Hymenoptera: Apidae). *PLoS One 3*, 1-6.

Hammer T and Menzel, R. (1995). Learning and memory in the honeybee. *J. Neurosci. 15*, 1617-1630.

Han P, Niu C-Y, Lei C-L, Cui J-J, and Desneux N. (2010). Use of an innovative T-tube assay and the proboscis extension response assay to assess sublethal effects of GM products and pesticides on learning capacity of the honey bee *Apis mellifera L. Ecotoxicol. 19*, 1612-1619.

Herrera C. (1990). Daily patterns of pollinator activity, differential pollinating effectiveness, and floral resource availability, in a summer-flowering Mediterranean shrub. *Oikos 58*(3), 277-288.

Pham-Delegue M, Bailez O, Blight M, Masson C, Picard-Nizou A, and Wadhams L. (1993). Behavioral discrimination of oilseed rape volatiles by the honeybee *Apis mellifera* L. *Chem. Senses 18*, 483-494.

Ramirez-Romero R, Desneux N, Decourtye A, Chaffiol A, and Pham-Delegue M H. (2008). Does Cry1Ab protein affect learning performances of the honey bee *Apis mellifera* (Hymenoptera, Apidae)? *Ecotoxicol. Environ. Safety 70*, 327-333.

Seeley T. (1985). Honeybee ecology. Princeton Univ. Press, Princeton.

Srinivasan M. (2010). Honey bees as a model for vision, perception, and cognition. *Annu. Rev. Entomol. 55*, 267-284.

Yu H-L, Li Y-H, and Wu K-M. (2011). Risk assessment and ecological effects of transgenic Bacillus thuringiensis crops on non-target organisms. *J. Integ. Plant Biol. 53*(7), 520-538.

**(b). Three commenters raised the issue of potential negative effects of *Bt* proteins on the monarch butterfly, specifically citing a 1999 publication by Losey et al.**

The commenter specifically referred to a laboratory study involving the exposure of monarch butterfly larvae to pollen from *Bt* corn (Losey et a., 1999). This research involved dusting milkweed plants (the host plant of monarch butterflies), with pollen from *Bt* corn. Pollen density had been set to visually match densities on milkweed leaves collected from corn leaves.

Exposed larvae ate less, grew more slowly, and suffered higher mortality than a control group of larvae. (Losey et al., 1999). Corn fields shed pollen for 8-10 days between late June and mid-August, when monarch larvae are feeding; 50% of monarch populations are concentrated around the corn belt in the U.S. Midwest (Losey et al., 1999). At the time, this work was taken as evidence that *Bt* harmed nontarget organisms.

However, later research cast doubt on the Losey et al. (1999) results. For example, Sears et al. (2001) conducted a "weight of evidence" two-year series of field trials in several states and in Canada. Their results suggested that the impact of *Bt* corn pollen on monarch populations is negligible. Sears et al. (2001) also criticized the Losey et al. (1999) report because Losey et al. did not specify the dosage of *B*t to which larvae had been exposed. Stanley-Horn et al. (2001) examined survival and growth of monarch larvae from exposure to 3 different *Bt* corn events (differing in toxin expression) in field studies. Although Stanley-Horn et al. (2001) indicated that the monarch butterfly is potentially at risk because milkweed grows in and near the edges of corn fields, their results showed only negligible effects on larvae. These results were bolstered by those of Wolt et al. (2003), who examined the effect of distance of host milkweed plants from the source of *Bt* corn. They found that pollen deposition from *Bt* corn onto milkweed plants declined exponentially with distance of plants from corn, and noted that the risk of mortality to monarch larvae is negligible on milkweed plants located >1 m from the edge of source corn fields.

*Bt* corn pollen did not increase mortality in a related species, the black swallowtail (Wraight et al. 2000), whose chief food plants occur in narrow strips between edges of corn fields and roads. The black swallowtail has potentially greater exposure to *Bt* corn pollen since it feeds on multiple plants near corn fields. The authors also cited other mortality causes which could contribute to lower abundance of larvae, such as predation (Wraight et al., 2000).

Prasifka et al. (2007) exposed monarch larvae to anthers (pollen-bearing organs) of *Bt* corn. Although they did find decreased feeding, body weight and movement in exposed larvae, these results are problematical since they found no evidence of actual feeding on the anthers, and did not cite any mechanisms for the effects found.

More recent review papers examining the weight of evidence of exposure of Lepidoptera to *Bt* found no negative results (Lang and Otto, 2010; Yu et al., 2011). Lang and Otto (2010) considered and reviewed only publications from peer-reviewed journals and which contained original data from lab or field studies that looked at direct toxic effects of *Bt* maize on nontarget lepidopteran larvae. They pointed out weaknesses of many previous studies, including: some laboratory experiments were often run under unrealistic conditions; *Bt* quantities were often not calculated; most studies only considered species within the superfamily Papilionoidea (to which the monarch and black swallowtail butterflies belong), even though other lepidopteran species are common in agricultural landscapes; some of the variables considered in studies were interrelated (not independent of each other); host plant quality, which could affect results, was

rarely considered; exposure period to Bt pollen was too short in some cases, less than what would be seen in field (Lang and Otto, 2010). They noted that negative effects were less frequently observed in field studies as opposed to those in the lab, which suggests that some of the positive results seen in lab studies may be artifacts of the experimental design. In another review of the effects of *Bt* crops on nontarget organisms, Yu et al. (2011) concluded that later research on toxicity of *Bt* crops to monarch larvae showed that risks were negligible because of limited exposure and toxicity of *Bt* corn pollen to monarchs.

Finally, 5307 corn expresses the eCry3.1Ab protein which demonstrates no toxicity toward lepidopteran insects, the order which includes butterflies (Syngenta, 2011c)

**References:**


Lang A and Otto M. (2010). A synthesis of laboratory and field studies on the effects of transgenic *Bacillus thuringiensis* (*Bt*) maize on non-target Lepidoptera. *Entomol. Exp. et Appl. 135*, 121-134.

Losey J, Raynor L, and Carter M. (1999). Transgenic pollen harms monarch larvae. *Nature 399*, 214.

Prasifka P, Hellmich R, Prasifka J, and Lewis L. (2007). Effects of Cry1Ab-expressing corn anthers on the movement of monarch butterfly larvae. *Environ. Entomol*. *36*(1), 228-233.

Sears M, Hellmich R, Stanley-Horn D, Oberhauser K, Pleasants J, Mattila H, Siegfried B, and Dively G. (2001). Impact of Bt pollen on monarch butterfly populations: a risk assessment. *Proceedings Nat. Acad. Sci. 98*(21), 11937-11942.

Stanley-Horn D, Dively G, Hellmich R, Mattila H, Sears M, Rose R, Jesse L, Losey J, Obrycki J, and Lewis L. (2001). Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. *Proceedings Nat. Acad. Sci. 98*(21), 11931-11936.

Syngenta. (2011). Syngenta Company petition for determination of nonregulated status of SYN-05307-1 rootworm resistant corn. http://www.aphis.usda.gov/biotechnology/not_reg.html)

Wolt J, Peterson R, Bystrak P, and Meade T. (2003). A screening level approach for nontarget insect risk assessment: transgenic *Bt* corn pollen and the monarch butterfly (Lepidoptera: Danaidae). *Environ. Entomol*. *32*(2), 237-246.

Wraight C, Zangerl A, Carroll M, and Berenbaum M. (2000). Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. *Proceedings Nat. Acad. Sci. 97*(114), 7700-7703.

Yu H-L, Li Y-H, and Wu K-M. (2011). Risk assessment and ecological effects of transgenic *Bacillus thuringiensis* crops on non-target organisms. *J. Integ. Plant Biol. 53*(7), 520-53.

**(c). Among the concerns raised about effect of Bt on nontarget organisms, three commenters referred to a 2012 Institute for Science in Society web posting, which describes a 2009 report by Schmidt et al (2009). The Schmidt paper suggests that exposure to *Bt* proteins led to increased mortality in the nontarget ladybird beetle predator *Adalia bipunctata*. Commenters also called attention to a 2012 paper (Hilbeck et al.) responding to critics of the Schmidt et al (2009) publication.**

**APHIS response:** Coccinellid (ladybird beetle) larvae are important predators of plant pests such as aphids, and can potentially be exposed to *Bt* through carnivory of the herbivores feeding on *Bt* crops (Rauschen et al., 2010). In the Schmidt (2009) study cited by the Institute for Science in Society website (Sirinathsinghji, 2012), toxicity was tested by spraying water containing the *Bt* toxins Cry1Ab and Cry3Bb on prey eggs of *Ephestia kuehniella*, the Mediterranean flour moth (Lepidoptera: Pyralidae), and offering them to *Adalia bipunctata*, the twospotted lady beetle (Coleoptera: Coccinellidae). Schmidt et al. (2009) reported that treatments using the Cry3Bb toxin (which is active on some beetles) did not produce statistically significant increases in mortality of lady beetles compared to control treatments. However, feeding lady beetles with lepidopteran-active Cry1Ab treated eggs produced statistically higher mortality than feeding beetles with control treated eggs. 5307 Corn produces the coleopteran-active Cry3Bb1 protein.

Several researchers have subsequently refuted the Cry1Ab results (Rauschen et al, 2010; Alvarez-Alfageme et al., 2011; Yu et al., 2011). The Schmidt study was weakened by poor design and methodology, which led to questions whether the observed *A. bipunctata* mortality resulted from *Bt* feeding or to some other source. Specifically, Schmidt et al. (2009) used a feeding bioassay in which *E. kuehniella* eggs were sprayed with water containing *Bt.* However, the *A. bipunctata* larvae (and other coccinellid larvae) mode of feeding involves piercing eggshells and sucking out the contents, not consuming the eggs whole, as was done in the Schmidt et al. (2009) research. Therefore, it is possible that the *A. bipunctata* larvae tested in the assay actually ingested insignificant amounts of Bt proteins. In addition, the mortality of larvae in the control group (21%) was very high, which suggests problems with the Schmidt et al. (2009) bioassay which may have contributed to only apparently increased mortality in larvae exposed to Cry1Ab (Alvarez-Alfageme et al., 2011; Yu et al., 2011). In addition, Rauschen et al.

(2010) and Alvarez-Alfageme et al. (2011) noted that the dosage of *Bt* used by Schmidt et al. (2009) was unreported, and remains unclear, so that Schmidt et al. (2009) did not define exposure, and therefore, level of risk before doing the experiment (Rauschen, 2010b). Under realistic field conditions, *A. bipunctata* larvae are exposed to low concentrations of *Bt* since their main prey item, aphids, consumes low amounts of *Bt* Cry proteins when feeding on *Bt* maize (Alvarez-Alfageme et al., 2011; Rauschen, 2010b) since *Bt*-maize does not carry Cry proteins in its phloem sap (Raps et al., 2001). Aphid predators are not likely to be exposed to *Bt* proteins from their prey under field conditions (e.g., Lundgren et al., 2005).

In order to provide more data on the effect of *Bt* crops on coccinellid (lady beetle) larvae, Alvarez-Alfageme et al. (2011) conducted another study on *A.bipunctata* larvae, but used spider mite (*Tetranychus urticae*) larvae as prey items instead of *Bt*-water sprayed eggs. The *T. urticae* larvae had previously fed on *Bt* maize. The results of this research demonstrated no negative effects of *Bt* on *A. bipunctata* larvae. Li and Romeis (2010) also showed that the protein found in Event 5307 corn, Cry3Bb1, does not harm spider mite or its ladybird beetle predator, *Stethorus punctillum*. An earlier paper (Al-Deeb and Wilde, 2003) investigated the effect of *Bt* corn expressing Cry3Bb1 toxin on foliar and ground-dwelling arthropods in Kansas over a two year period. Specifically, Al-Deeb and Wilde (2003) examined the effect of *Bt* for corn rootworm control on the coccinellids *Coleomegilla maculata* (spotted lady beetle), *Hippodamia convergens* (convergent lady beetle) and *Scymnus* spp. lady beetles, but found no significant differences between numbers of these beetles and control groups of the same beetle species exposed to non-*Bt* corn.

In a response to critics of the Schmidt et al. (2009) paper, Hilbeck et al. (2012) rejected charges that differences between the Schmidt et al. (2009) paper and others were due to differences in experimental protocol, and, in turn, criticized the arguments of the detractors (e.g., Rauschen, 2010; Alvarez-Alfageme et al., 2011) Hilbeck et al. (2012) changed protocols for a new set of observations which they stated corroborated the original results of Schmidt et al. (2009). APHIS notes that Hilbeck was also an author on the Schmidt et al (2009) paper, so is not unbiased. APHIS concludes, however, that the weight of the evidence confirms that *Bt* is not toxic to ladybird beetle (coccinellid) populations, (see e.g., Alvarez-Alfageme et al., 2008, Bhatti et al., 2005; and Ahmad et al., 2006).

**References**

Ahmad A, Wilde G, Whitworth R, and Zolnerowich G. (2006). Effect of corn hybrids expressing the coleopteran-specific Cry3Bb1 protein for corn rootworm control on above-ground predators. *J. Econ. Entomol. 99*,1085-1095.

Al-Deeb M and Wilde G. (2003). Effect of *Bt* corn expressing the Cry3Bb1 toxin for corn rootworm control on above ground nontarget arthropods. *Environ. Entomol. 32*(5), 1164-1170.

Alvarez-Alfageme F, Bigler F, and Romeis J. (2011). Laboratory toxicity studies demonstrate no adverse effects of Cry1Ab and Cry3Bb1 to larvae of *Adalia bipunctata* (Coleoptera: Coccinellidae): the importance of study design. *Transgenic Res. 20*, 467-479.

Alvarez-Alfageme F, Ferry N, Castanera P, Ortego F, and Gatehouse A. (2008). Prey mediated effects of Bt maize on fitness and digestive physiology of the red spider mite predator *Stethorus punctillum* Weise (Coleoptera: Coccinellidae). *Transgenic Res. 17*, 943- 954.

Bhatti M, Duan J, Head G, Jiang C, McKee M, Nickson T, Pilcher C, and Pilcher C. (2005). Field evaluation of the impact of corn rootworm (Coleoptera: Chrysomelidae) protected *Bt* corn on foliage-dwelling arthropods. *Environ. Entomol. 34*, 1336-1345.

Hilbeck A, McMillan J, Meier M, Humbel A, Schlapfer-Miller J, and Trtikova M. (2012). A controversy revisited: is the coccinellid *Adalia bipunctata* adversely affected by *Bt* toxins? *Environ. Sci. Europe 24*, 10.

Li Y and Romeis J. (2010). *Bt* maize expressing Cry3Bb1 does not harm the spider mite, *Tetranychus urticae*, or its ladybird beetle predator, *Stethorus punctillum*. *Biol. Control 53*, 337-344.

Liu B, Shu C, Xue K, Zhou K, Li X, Liu D, Zheng Y, and Xu C. (2009). The oral toxicity of the transgenic Bt+CpTI cotton pollen to honeybees (*Apis mellifera*). *Ecotoxicol. Environ. Safety 72*, 1163-1169.

Lundgren J. and Wiedemann R. (2005). Tritrophic interactions among *Bt* (Cry3Bb1) corn, aphid prey, and the predator *Coleomegilla maculata* (Coleoptera: Coccinellidae). *Environ. Entomol. 34*, 1621-1625.

Raps A, Kehr J, Gurgli P, Moar W, Bigler F, and Hilbeck A. (2001). Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and the nontarget herbivore *Rhodalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. *Mol. Ecol. 10*, 525-533.

Rauschen S. (2010). A case of "pseudo science"? A study claiming effects of the Cry1Ab protein on larvae of the two-spotted ladybird is reminiscent of the case of the green lacewing. *Transgenic Res. 19*, 13-16.

Schmidt J, Braun C, Whitehouse L, and Hilbeck A. (2009). Effects of activated *Bt* transgene products (Cry1Ab, Cry3Bb) on immature stages of the ladybird *Adalia bipunctata* in laboratory ecotoxicity testing. *Arch. Environ. Contam. Toxicol. 56*, 221-228.

Sirinathsinghji E. (2012). Bt toxicity confirmed: flawed studies exposed. Retrieved from Institute for Science in Society.website. Accessed October 31, 2012. http://www.isis.org.uk/Bt_Toxicity_Confirmed_Flawed_Study_Exposed.php.

Yu H-L, Li, Y-H, and Wu K-M. (2011). Risk assessment and ecological effects of transgenic *Bacillus thuringiensis* crops on non-target organisms. *J. Integ. Plant Biol. 53*(7), 520-538.

**(d). Three commenters expressed concern over the impact of *Bt* on health of livestock which might browse on *Bt* fields and noted three website reports which purport to show that *Bt* is injurious to cattle and goats (Greenpeace, 2003; Ramdas, 2010; Srinigathsinghji, 2012).**

**APHIS response:** APHIS reviewed the website reports (Greenpeace, 2003; Ramdas, 2010; Srinigathsinghji, 2012) cited by commenters. All of these reports center on anecdotal instances of possible exposure of livestock or farm animals (sheep, goats, and cattle) to *Bt* crops, and the perception that the animals later became ill and/or died as a direct result. The papers attribute, with no supporting information or data, these illnesses and mortality to *Bt* crop (cotton and maize) feeding. The authors did not propose or examine any other potential causes of morbidity and mortality. The Greenpeace report about cattle in Germany (2003) also mentioned some alleged sublethal effects such as less milk produced by cattle exposed to *Bt* maize, but presents no further information or data to suggest that their deaths and/or lowered milk production were, in fact, caused by consumption of *Bt* in feed.

There is ample evidence in the literature that *Bt* crops are safe for farm animals (e.g., Faust, 2002; Konig et al, 2004; Flachowsky et al., 2005; Shimada et al., 2008; and Hartnell, 2010). For example, Guertler et al. (2010) tested the effects of *Bt* maize on dairy cows, and found no differences in the composition of their milk compared with the milk of a control group of cows that had been fed conventional maize. Steinke et al. (2010) also fed *Bt* corn to dairy cattle, but found no consistent effects on the animals. Iphaguerre et al. (2003) fed dairy cows with silage containing *Bt*, and determined that for lactating dairy cows, the chemical composition of the feed was not altered, nor was nutritional value diminished compared with conventional corn feeds.

Walsh et al. (2011) fed GE maize to weanling pigs, and found that there were no negative effects on growth of animals or on body weight. Walsh et al. (2011) also looked at the immune response. While they found some increase in immune response, they reported that its "biological relevance is questionable," citing other physiological reasons not related to *Bt* ingestion which might account for the increased response. Buzioaneau et al. (2012) fed transgenic maize to gestating and lactating sows to determine the effect of Bt on maternal and offspring immunity. They reported that although they found Cry1Ab in sows' blood and feces approximately four months after onset of the experiment, and in blood and tissues of offspring at birth, *Bt* maize did

not represent any significant immunological challenges to the treated pigs. The effects "did not indicate inflammation or allergy and are unlikely to be of major importance." Buzioaneu et al. (2012) concluded that their findings lent further support to the safety of *Bt* maize.

Trabalza-Maranucci et al. (2008) fed *Bt*176 maize to sheep over a period of three years, and found no negative effects on animal health, nor was any Bt DNA found in the animals' tissues, blood, or ruminal bacteria. This paper emphasizes the advantages of conducting long-term experiments where possible in order to study cause and effects.

The US FDA (2012) has also examined studies of broiler chickens fed with Syngenta 5307 corn and that fed with near isogenic corn, and agrees with Syngenta's conclusion that there are no differences between Syngenta corn and commercial corn in terms of impacts on livestock. No compositional differences were detected between Syngenta 5307 corn and other similar varieties.

Similar to the regulatory control for direct human consumption of corn under the FFDCA, it is the responsibility of feed manufacturers to ensure that the products they market are safe and properly labeled. Feed derived from GE corn must comply with all applicable legal and regulatory requirements, which in turn protects human health. Syngenta completed the consultation process with FDA for Event 5307 corn on February 29, 2012, establishing the safety of Syngenta 5307 corn for food and feed use. EPA has granted an exemption from food and feed tolerance for the phosphomannose isomerase (PMI) protein on April 25, 2007 and the eCry3.1Ab protein on August 8, 2012.

**References**:


Buzoianeau S, Walsh M, Rea M, O'Donovan O, Gelencser E, Ujhelyi G, Szabo E, Nagy A, Ross R, Gardiner G, and Lawlor P. (2012). Effects of feeding *Bt* maize to sows during gestation and lactation on maternal and offspring immunity and fate of transgenic material. *PLoS One 7*(10, e47851.

Faust M. (2002). New feeds from genetically modified plants: the U.S. approach to safety for animals and the food chain. *Livestock Prod. Sci. 74*(3), 239-254.

Flachowsky G, Chesson A, and Aulrich K. (2005). Animal nutrition with feeds from genetically modified plants. *Arch. Anim. Nutr*. *59*(1), 1-40.

Greenpeace. (2003). Is GM maize responsible for cow deaths in Hesse? Retrieved from http://www.gene.ch/genet/2003/Dec/msg00152.html/. Accessed October 30, 2012.

Guertler P, Paul V, Steinke K, Wiedemann S, Preisinger W, Albrecht C, Spiekers C, Schwarz F, and Meyer H. (2010). Long-term feeding of genetically modified corn (MON810) –fate of Cry1Ab DNA and recombinant protein during the metabolism of the dairy cow. *Livestock Sci. 131*(2-3), 250-259.

Hartnell G. (2010). Feeding transgenic feedstuffs to cattle. University of Florida. Retrieved from dairy.ifas.ufl.edu/ms/2010/7-Hartnell.pdf. Accessed November 4, 2012.

Ipharraguerre I, Younker R, Clark J, Stanisiewski E, and Hartnell G. (2003). Performance of lactating dairy cows fed corn as whole plant silage and grain produced from a glyphosate-tolerant hybrid (event NK603). *J. Dairy Sci. 86*, 1734-1741.

Konig A, Cockburn A, Crevel R, Debruyne E, Grafstroem R, Hammerling U, Kimber I, Knudsen I, Kuiper H, Peijnenburg A, Poulsen M, Schauzu M, and Wal J. (2004). Assessment of the safety of foods derived from genetically modified (GM) crops. *Food Chem. Toxicol*. *42*(7), 1047-1088.

Ramdas S. (2010). National workshop on genetically modified crops/foods and health impacts, Centre for Sustainable Agriculture, Doctors for Food and Bio-safety, Greenpeace India and Sustainet. Retrieved from http://www.gmwatch.eu/latest-listing/1-news-items/11872-bt-cotton-and-livestockhealth-impacts-dr-sagari-r-ramdas. Accessed on October 31, 2012.

Shimada N, Murata H., Mikami Initial and Miyazaki Initial. (2008). Safety evaluation of *Bt* plants: review. *Jap. Agric. Res. Quart. 42*(4), 251-259.

Sirinathsinghji, E. (2012). Syngenta charged for covering up livestock deaths from GM corn. Retrieved from Institute for Science in Society website. Retrieved from http://www.i-sis.org.uk/Syngenta_Charged_for_Covering_Up_Livestock_Deaths_from GM_Corn.php. Accessed November 5, 2012.

Steinke K, Guertler P, Paul V, Wiedemann S, Ettle L, Albrecht C, Meyer H, Spiekers H, and Schwarz F. (2010). Effects of long-term feeding of genetically modified corn (event MON810) on the performance of lactating dairy cows. *J. Anim. Physiol. Nutr. 94*, e185-193.

Trabalza-Marinucci M, Brandi G, Rondini C, Avellini L, Giammarini C, Costarelli S, Acute G, Orlandi C, Filippini G, Chiaradia E, Malatesta M, Crotti S, Antonini C, Amagliani G, Manuali E, Mastrogiacomo A, Moscati L, Haouet M, Gaiti A, and Magnani M. (2008). A three-year longitudinal study on the effects of a diet containing genetically modified *Bt*176 on the health status and performance of sheep. *Livestock Sci. 113*, 178-190.

US FDA (2012). Biotechnology Consultation Note to the File BNF No. 000128. Event 5307 Coleopteran insect-resistant corn expressing eCry3.1Ab. U.S. Food and Drug Administration. http://www.fda.gov/Food/Biotechnology/Submissions/ucm304082.htm

Walsh M, Buzoianu S, Gardiner G, Rea M, Gelencser E, Janosi A, Epstein M, Ross R, and Lawlor P. (2011). Fate of transgenic DNA from orally administered *Bt* MON810 maize and effects on immune response and growth in pigs. *PLoS One 6*(11), e27177.

**Comment 5: Four comments described concerns that *Bt* is detrimental to human health, pointing to the following references: Noble et al. (1992), Vasquez (1999a, 1999b, 2000), EPA (2000); GMWatch (2004), Espada (2004), Ho (2006), Aris and Leblanc (2011) and Mesnage et al (2012).**

**APHIS response:** The commenter appears to have misunderstood the Vasquez publications (1999a, 1999b, 2000) as demonstrating that *Bt* has negative effects on human health. These publications described induction of immune response in mice which were immunized with a solution of buffer and Cry1Ac protein. The authors then discussed the potential for use of Cry proteins in the development of cheap and effective vaccines for animals and humans, given that it is "innocuous to vertebrates" (Vasquez et al., 1999a; Vasquez et al., 1999b;Vasquez-Padron et al., 2000). Vasquez et al. (2000) reported that the Cry1Ac protein, when fed to mice, induced an immunological reaction, including the production of antibodies. Mice were immunized with solutions of purified Cry1Ac proteins and buffer (as an antigen).and indirectly measured induction of a mucosal immune response in fresh feces from immune mice. The presence of antibodies is frequently associated with inflammation; however, innocuousness of *Bt* to vertebrates is well documented (McClintock et al., 1995).

Commenters also referred to an EPA report on *Bt* risks and benefits (2000). This report stated that it Cry1Ac proteins are "unlikely to have significant adverse ecological effects on populations of wild mammals, birds, non-arthropodan invertebrates, and aquatic species". Regarding *Bt* effects on human health, the EPA in 2000 recommended that acute and chronic exposure to *Bt* studies should be performed.

Mesnage et al. (2012) tested the effects of a combination of the *Bt* toxins Cry1Ab and Cry1Ac and glyphosate residues on biomarkers of human cell death on a human kidney cell line. They reported that although Cry1Ac caused no toxicity to cells, Cry1Ab did. Mesnage et al. (2012) argue that a combination of *Bt* and glyphosate residues from genetically modified plants may cause side effects on humans. However, this research appears to be a preliminary study on a specific cell line. Under the Coordinated Framework, FDA has the responsibility of reviewing human health issues, and setting tolerances for compounds in foods. Additionally, cell exposure in vivo to these chemicals would not resemble either qualitatively or quantitatively whole animal ingestion and so this report is not relevant.

Although the Noble et al. (1992) report was described by a commenter as demonstrating evidence that *Bt* has negative effects on human health, the opposite is true. The report describes surveys of potential human health effects on residents of a region of British Columbia, Canada

following a 1992 combined aerial and ground spray program to control Asian gypsy moth, using a product, Foray 48B, whose active ingredient is *Btk* (*Bacillus thuringiensis kurstaki*), a microbial insecticide routinely used in forest gypsy moth control. The report combined the results of medical professionals, emergency departments in hospitals, and worker exposure, and found no significant negative effects on human health. Although workers were occupationally exposed to *Btk*, generally at much higher levels than residents living near the spray zone, Noble et al. (1992) concluded that even worker health effects were negligible. Bacterial cultures of some individuals who visited hospitals for a variety of complaints sometimes tested positive for *Btk.* However, the authors made the assignment of positive cultures based on bacterial colony morphology, such as crystals and spores, but did not measure or otherwise quantitate *Btk* concentration. Similarly, no analysis of blood samples was conducted to measure *Btk* concentration in human blood samples was reported. Moreover, the authors sampled fresh fruits and vegetables from organic and conventional grocery stores, and detected levels of *Btk*, suggesting that residents were exposed to *Btk* by the consumption of these foods.

Three of the references cited by commenters as relevant were website entries (GMWatch/Traavik 2004; Espada, 2004; and Ho, 2006). All of them refer to findings of Prof. Traavik, a professor at the University of Tromso in Norway, who said he found the presence of antibodies to *Bt* (Cry1Ab) proteins in the blood of 38 people in the Philippines, who were living near a field of *Bt* maize. Ho (2006) also reported that the Filipino villagers became ill, as well as livestock, allegedly due to exposure to the *Bt* proteins. None of the website entries are referenced and substantiated with any other data. No evidence linking any alleged effects with *Bt* proteins was presented.

In the study by Aris and Leblanc (2011) on the effect of *Bt* on maternal and fetal health, the Cry1ab protein (a common insecticidal protein introduced into GE crops such as corn) was detected in 93 percent of maternal blood, 80 percent of fetal blood, and 69 percent of blood from non-pregnant women. The subjects of this study all resided in Sherbrooke, an urban area of Eastern Townships of Quebec, Canada. While Aris and Leblanc (2011) detected the Cry1ab protein in the majority of blood samples tested, the authors did not make any effort to determine the origin of the Cry1ab protein, only assuming that the source of Cry1ab must be through the consumption of GE crops, "given the widespread use of GM [GE] foods in the local daily diet (soybeans, corn, potatoes), it is conceivable that the majority of the population is exposed through their daily diet." However, the authors neglect to mention that *Bacillus thuringiensi*s, a bacterium from which Cry1ab is derived and produced, is commonly used in organic farming (either as protein sprays or spray of the *B. thuringiensis* itself) (Aroian, 2011; EPA, 2005). In previous studies, naturally-occurring *B. thuringiensis* has been detected in fresh fruits and vegetables (Frederiksen et al., 2006), milk, ice cream, and green tea samples (Zhou et al., 2008); and human nasal samples following aerial sprays to control gypsy moth populations (Valaderes de Amorim et al., 2001.)

Additionally, Aris and Leblanc (2011) made no effort to eliminate the probability of detecting false positives through the ELISA-based screening kit (DAS ELISA kit for *Bt*-Cry1ab/Ac protein, Agdia). The detection limit for the DAS ELISA kit for *Bt*-Cry1ab/1Ac protein is reported to be 1 ng/ml (Paul et al., 2008); however, Aris and Leblanc detected the Cry1qb protein at averaged levels of approximately 0.18 ng/ml in the blood serum of pregnant women, 0.12 ng/ml in the blood serum of non-pregnant women, and 0.05 ng/ml in the blood serum of human fetuses. The 1 ng/ml detection limit of the ELISA kit and the levels detected in the study is problematic, as the detection limit of a kit is generally regarded as the lowest possible level for which a user may reliably detect a compound. Unfortunately, no additional Cry1ab protein detection method was cited in the Aris and Leblanc (2011) study to corroborate and verify that these very low detection levels did not constitute false positives, as would be standard practice. With regard to the ELISA kit itself, it was not validated for its suitability to measure Cry1ab in human blood; rather, it was designed to detect Cry1ab extracted from plant tissues (Agdia, 2011; FSANZ, 2011).

APHIS also disagrees with the implication that *Bt* proteins (Cry family proteins) are inherently dangerous to human health. APHIS directs commenters to previous EAs (USDA-APHIS, 2011) that have examined the risk of human exposure to *Bt* proteins and determined that *Bt* proteins pose little risk to human health.

In summary, APHIS believes that the study of Aris and Leblanc (2011) has several shortcomings that bring its conclusions about the detection of the Cry1ab protein into doubt. These include issues surrounding the source of the Cry1ab detected, problems with the assay method used to detect the Cry1ab protein, and the implication that Cry1ab poses any significant risk to human health.

**References:**


Agdia (2011). DAS Elisa for the detection of *Bt*-Cry1ab/1ac proteins. Agdia. Retrieved from https://orders.agdia.com/Documents/m172.pdf. Accessed December 20, 2012.

Aroian R. (2011). *Bacillus thuringiensis*—organic farming. University of California San Diego. Retrieved from http://www.bt.ucsd.edu/organic_farming.html. Accessed November 6, 2012

Aris A and Leblanc S. (2011). Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. *Reprod. Toxicol.*, *31*(4): 528-533.

EPA Scientific Advisory Panel, *Bt* Plant-Pesticides Risk and Benefits Assessments, March 12, 2001: 76. Retrieved from

http://www.epa.gov/scipoly/sap/meetings/2000/october/octoberfinal.pdf. Accessed October 31, 2012.

Espada D. (2004). Independent probe shows Bt-corn has human costs. Retrieved from http://www.bulatlat.com/news/4-7/4-7-btcorn.html. Accessed October 31, 2012.

Frederiksen K, Rosenquist H, Jorgensen K, and Wilcks A. (2006). Occurrence of natural *Bacillus thuringiensis* contaminants and residues of *Bacillus-thuringiensis*-based insecticides on fresh fruits and vegetables. *Appl. Environ. Microbiol. 72*(5), 3435-3440.

FSANZ. (2011). FSANZ response to study linking Cry1ab proteins in blood to GM foods. Food Standards Australia and New Zealand. Retrieved from http://www.foodstandards.gov.au/consumerinformation/gmfoods/fsanzresponsetostudy5185.cfm. Accessed December 20, 2012.

GMWatch. (2004). Prof. Terje Traavik in his own words. Retrieved from http://www.gmuwatch.org/latest-listing/1-news-items/6077-prof-terje-traavik-in-his-own-words. Accessed November 6, 2012.

Ho M-H. (2006). GM ban long overdue. Institute of Science in Society website. Retrieved from www.i-sis.org.uk/GMBanLongOverdue.php. Accessed December 20, 2012.

McClintock J, Schaffer C, and Sjoblad R. (1995). A comparative review of the mammalian toxicity of *Bacillus-thuringiensis* based pesticides. *Pesticide Sci. 45*, 95-105.

Mesnage R, Clair E, Gress S, Then C, Szekacs A, and Seralini G-E. (2012). Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. *J. Appl. Toxicol.* DOI 10.1002/jat.2712.

Noble M, Riben P and Cook G. (1992). Microbiological and epidemiological surveillance program to monitor the health effects of Foray 48B *Btk* spray. Vancouver, B.C.: Ministry of Forests, Province of British Columbia.

NRC. (2010). Impact of genetically engineered crops on farm sustainability in the United States. The National Academies Press, Washington, D.C.

Valaderes de Amorim G, Whittome B, Shore B, and Levin D. (2001). Identification of *Bacillus thuringiensis* subsp. *kurstaki* strain Hd1-like bacteria from environmental and human samples after aerial spraying of Victoria, British Columbia, Canada, with Foray 48b. *Appl. Environ. Microbiol.*, *67*(3), 1035-1043.

Vazquez-Padron R, Moreno-Fierros L, de la Riva G, and Lopez-Revilla R. (1999). Intragastric and intraperitoneal administration of Cry1Ac protoxin from *Bacillus*

*thuringiensis* induces systemic and mucosal antibody responses in mice. *Life Sci.*, *64*(21), 1897–1912.

Vazquez R, Moreno-Fierros L, Neri-Bazan L, de la Riva G, and Lopez-Revilla R. (1999). *Bacillus thuringiensis* Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scand. *J. Immunol. 49*, 578–584.

Vazquez-Padron R, Moreno-Fierros L, Neri-Bazan L, Martinez-Gil A, de la Riva G, and Lopez-Revilla R. (2000). Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from *Bacillus thuringiensis* HD 73 in mice. *Braz. J. Med. Biol. Res. 33*, 147–155.

Walsh M, Buzoianu S, Gardiner G, Rea M, Gelencser E, Janosi A, Epstein M, Ross R, and Lawlor P. (2011). Fate of transgenic DNA from orally administered *Bt* MON810 maize and effects on immune response and growth in pigs. *PLoS One 6*(11), e27177.

Wu F. (2006). An analysis of *Bt* corn's benefits and risks for national and regional policymakersconsidering Bt corn adoption. *Internat. J.Technol.Globalisation, 2*(1), 115-136.

Yu, H-L, Li Y-H, and Wu K-M. (2011). Risk assessment and ecological effects of transgenic *Bacillus thuringiensis* crops on non-target organisms. *J. Integ. Plant Biol. 53*(7), 520-53

**Comment 6: One commenter stated that there is a lack of testing for human or environmental safety**

**APHIS response:**

The Coordinated Framework, published by the Office of Science and Technology Policy (51 FR 23302, 57 FR 22984) describes the comprehensive federal regulatory policy for ensuring the safety of biotechnology research and products and explains how federal agencies will use existing Federal statutes in a manner to ensure public health and environmental safety while maintaining regulatory flexibility to avoid impeding the growth of the biotechnology industry. The U.S. Food and Drug Administration (FDA) regulates GE organisms under the authority of the Federal Food, Drug, and Cosmetic Act (FFDCA). The FDA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those that are genetically engineered. To help developers of food and feed derived from GE crops comply with their obligations under Federal food safety laws, FDA encourages them to participate in a voluntary consultation process. All food and feed derived from GE crops currently on the market in the United States have successfully completed this consultation process. The FDA policy statement concerning regulation of products derived from new plant varieties, including

those genetically engineered, was published in the *Federal Register* (FR) on May 29, 1992 (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 has provided the FDA with information on the identity, function, and characterization of the genes, including expression of the gene products. The submittal to the FDA included safety and nutritional assessment of food and feed derived from SYN-05307-1 to the FDA in January 2011 (Syngenta, 2011). Syngenta completed the consultation process with FDA for Event 5307 corn on February 29, 2012 and demonstrated that the 5307 corn was safe for food and feed.

Human health effects have not been identified from consuming the novel proteins introduced into *Bt* corn. The US-EPA requires seed registrants to submit tests of potential toxicity and allergenicity of the transgenic proteins in *Bt* corn cultivars before they can be approved for human consumption. All tests that have been performed for adverse mammalian impact from ingesting Cry proteins have been negative, even at extremely high doses (Wu, 2006). In addition, the toxicity of insecticidal *Bt* proteins depends on binding to specific receptors present in the insect midgut (e.g., Yu et al., 2011). EPA must provide a tolerance for the presence of transgenic expression of new proteins in crop products. In response to the request made by Syngenta, the EPA has granted an exemption from food and feed tolerance for the phosphomannose isomerase (PMI) protein on April 25, 2007 and the eCry3.1Ab protein on August 8, 2012.

As discussed in Section 5 of the EA, based on APHIS' review of field and laboratory data and scientific literature provided by Syngenta (Syngenta, 2011) and safety data available on other GE corn, APHIS has concluded that a determination of nonregulated status of 5307 Corn would have no significant impacts on human health.

As discussed in Section 5 of the EA, APHIS has concluded that a determination of nonregulated status of 5307 Corn would have no significant impacts on animal feed or animal health. Syngenta has submitted compositional and nutritional characteristics of 5307 Corn to APHIS (Syngenta, 2011). APHIS has reviewed Syngenta's results and has concluded that the levels of nutrients, anti-nutrients, and secondary metabolites in 5307 Corn are not statistically different from those likely to be expressed by conventional varieties.

As noted by the National Research Council (NRC), unexpected and unintended compositional changes arise with all forms of genetic modification, including both conventional hybridizing and genetic engineering (NRC, 2004). The NRC also noted at the time, no adverse health effects attributable to genetic engineering had been documented in the human population. Reviews on the nutritional quality of GE foods have generally concluded that there are no significant nutritional differences in conventional versus GE plants for food or animal feed (Faust, 2002; Flachowsky et al., 2005).

**References:**

Faust M. (2002). New feeds from genetically modified plants: the U.S. approach to safety for animals and the food chain. *Livestock Prod. Sci*. *74*(3), 239-254.

Flachowsky G, Chesson A, and Aulrich K. (2005). Animal nutrition with feeds from genetically modified plants. *Arch. Anim. Nutr. 59*(1), 1-40.

NRC. (2004). Safety of Genetically Engineered Foods: Approaches to Assessing Unintended Health Effects. Report, Washington, D.C., National Research Council.

Syngenta. (2011). Syngenta Company Petition for Determination of Nonregulated Status of SYN-05307-1 Corn Rootworm Resistant Corn. Submitted by D. Ward, Regulatory Affairs Manager. (See Table http://www.aphis.usda.gov/biotechnology/not_reg.html).

Wu F. (2006). An analysis of Bt corn's benefits and risks for national and regional policymakers considering Bt corn adoption. *Internat. J.Technol. Globalisation 2*(1):115-136.

Yu, H.-L., Li, Y.-H., and Wu, K.-M. (2011). Risk assessment and ecological effects of transgenic Bacillus thuringiensis crops on non-target organisms. J. *Integ. Plant Biol. 53*(7): 520-53

**Comment 7: One commenter asserted that Syngenta has not thus far been successful in obtaining sufficient authorizations to import 5307 corn. The commenter states that failure to obtain the authorizations in key markets within the world would create a risk of significant economic losses to U.S. grain and oilseed producers and markets.**

**APHIS response:**

The trade economic environment would not be affected as a direct or indirect result of the deregulation of 5307 Corn. A determination of non-regulated status of 5307 Corn would provide growers with an alternative to other transgenic corn rootworm-protected varieties that are currently available. Worldwide market conditions and destination country approval of transgenic crop commodities would continue to be factors for international corn prices, without regard to the presence or absence of 5307 Corn on the market. A determination of non-regulated status of 5307 Corn would not adversely impact the trade economy and may potentially enhance it through more efficient production of corn supplies worldwide.

To avoid adversely affecting international trade in corn commodities exported from the US (and Canada), Syngenta has applied to the following countries for cultivation approval or importation of 5307 Corn: Australia (import, approved April 29, 2012), U.S. EPA (cultivation, registered July 31, 2012), U.S. FDA (cultivation, under review with public comment period completed), USDA (cultivation, under review, public comment period completed), Canada-Food (cultivation, under review), Canada-Feed (cultivation, under review), Canada-Environment (cultivation, under review), Mexico (import, under review), Japan-Environment (import, under review, public

comment period completed), Japan-Food (import, under review), Japan-Feed (import, under review), Korea-Environment (import, under review), Korea-Food (import, under review), Philippines (import, under review), Thailand (import, under review), Taiwan (import, under review), China (import, USDA deregulation is needed for submission), EU (import, under review), Russia (import, under review), and Colombia (import, under review). When international acceptance of a specific event has not been attained, US elevators and grain buyers may either refuse to purchase the grain, or may require that it be diverted to elevators that are solely designated as sources for domestic grain sale (Reuters, 2011).

**Reference:**

Reuters. US Edition (2011). Cargill bars Syngenta corn variety at US wet mills. Thursday, September 1, 2011. http://www.reuters.com/article/2011/09/01/cargill-corn-idUSN1E78017Q20110901