Finding of No Significant Impact
Animal and Plant Health Inspection Service
Petition for Non-regulated Status for Corn Line MIR604 (APHIS 04-362-01p)

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared a revised, final environmental assessment (EA) prior to making its determination of whether or not to approve a petition (APHIS number 04-362-01p) for a determination of nonregulated status received from Syngenta Seeds, Inc., under APHIS regulations at 7 CFR Part 340. The subject of this petition, corn (*Zea mays* L.) line MIR604, is genetically engineered to express a modified *Bacillus thuringiensis* Cry3A (mCry3A) protein that selectively controls corn rootworm species. On January 10, 2007, APHIS published a notice in the *Federal Register* (72 *FR* 1212-1214, Docket no. 2006-0157) announcing the availability of the draft EA for public review and comment for a 30-day comment period, ending February 9, 2007, which was extended to close on March 9 (72 *FR* 7952), and the availability of the subject petition for public review and comment for a 60-day comment period, ending March 12, 2007. APHIS received 14 comments regarding the EA and 27 comments regarding the petition. APHIS' responses to the issues raised during the comment periods are included as an attachment to this document.

In the EA, APHIS considered three alternatives: Alternative A – No Action: Continuation as a Regulated Article; Alternative B – Determination that MIR604 corn is No Longer a Regulated Article, in Whole; Alternative C – Determination that MIR604 corn is No Longer a Regulated Articles, in Part. APHIS proposed Alternative B as its preferred alternative because of the lack of plant pest characteristics displayed by the MIR604 corn. Based upon analysis described in the revised, final EA and in APHIS' response to comments, APHIS has determined that the preferred alternative, to grant the petition in whole, will not have a significant impact on the quality of the human environment because:

1. There would be no significant environmental impact as a result of gene introgression from this corn line. In assessing the potential risks associated with gene introgression from MIR604 corn into its sexually compatible relatives, APHIS considered two primary issues: a) the potential for gene flow and introgression; and b) the potential impact of introgression. While all teosinte members can be crossed with cultivated corn to produce fertile hybrids, hybridization in nature is extremely unlikely because the distributions of teosinte and *Z. mays* do not overlap (<http://www.maizegenetics.net/index.php?page =domestication/taxonomydistribution.html>), and because of differences in developmental morphology and reproductive timing between the two species. First-generation corn-teosinte hybrids are generally less fit for survival and dissemination, and they show substantially reduced reproductive capacity. Therefore, it is very unlikely that gene introgression into a wild corn relative will occur. In addition, the introgression of the pesticidal gene or the phosphomannose isomerase (*pmi*) gene into a wild corn relative would not be expected to cause it to become a weed or cause any other indirect environmental effects. None of the sexually compatible relatives of corn in the U.S. are considered weeds in the U.S., and years of APHIS experience with *Bacillus thuringiensis* proteins expressed in plants have demonstrated that the agricultural use of these proteins does not lead to the development of new weeds or other effects. Therefore, there would be no impact related to outcrossing by deregulating this line in whole (Alternative

B). There would also be no impact from continuing to regulate the line (Alternative A) or to deregulate in part (Alternative C).

2. *Z. mays* does not persist in unmanaged ecosystems. No data of which APHIS is aware indicate that the presence of the *mCry3A* gene and the *pmi* gene improves the ability of this corn variety to survive without human intervention, nor is there any foreseeable reason to conclude that these two genes would affect this variety's survival in the wild. Therefore, because corn can only survive when it is cultivated and managed with existing cultural practices, there would be no weed impact from granting nonregulated status in whole to this genetically engineered variety (Alternative B) and its subsequent release relative to the release of any conventional corn variety. There would also be no impact from continuing to regulate the line (Alternative A) or to deregulate in part (Alternative C).

3. APHIS does not expect MIR604 to have any impacts on non-target organisms, including beneficial organisms and threatened or endangered species, because the insecticidal activity of the mCry3A protein is limited to target pest species, namely corn rootworm. Based on the specificity of the mCry3A activity, species outside the order Coleoptera and family Chrysomelidae should not be affected. Further, analysis of federally-listed threatened and endangered species (TES) and species proposed for listing revealed no TES within the family Chrysomelidae. Furthermore, two identified threatened and endangered beetles (Order: Coleoptera), namely the American burying beetle and Hungerford's crawling water beetle, either do not occur in corn fields or they inhabit areas where they will not be exposed to the mCry3A protein. The *pmi* gene is not known to have any toxic properties. Therefore, there would be no impact to non-target organisms, including beneficial organisms and threatened or endangered species, by deregulating this line in whole (Alternative B). There would also be no impact from continuing to regulate the line (Alternative A) or to deregulate in part (Alternative C).

4. Analysis of available information indicates that MIR604 exhibits no traits that would cause increased weediness and that its unconfined cultivation should not lead to increased weediness of other cultivated corn. MIR604 line exhibited no changes in disease susceptibility, and years of APHIS experience with *Bacillus thuringiensis* proteins expressed in plants have demonstrated that the agricultural use of these proteins does not lead to the harm of non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service. Based on this analysis, there is no apparent potential for significant impact to biodiversity by deregulating this line in whole (Alternative B). There would also be no impact from continuing to regulate the line (Alternative A) or to deregulate in part (Alternative C).

5. If MIR604 were to be grown commercially, the effect on agricultural practices from introducing MIR604 into the environment would be no different than for other deregulated corn lines expressing Cry proteins from *Bacillus thuringiensis*, with which APHIS has years of experience. Because the level of insect resistance in MIR604 is similar to previously deregulated genetically engineered corn varieties, there should be no difference in impact on standard agricultural practices in corn cultivation and controlling volunteer corn. With respect to impacts on organic farmers, MIR604 corn should also not present new and different issues than currently available deregulated corn varieties expressing Cry proteins, nor should MIR604 significantly

impact organic production. Based on this analysis, there is no apparent potential for significant impact on standard agricultural practices by deregulating this line in whole (Alternative B). There would also be no impact from continuing to regulate the line (Alternative A) or to deregulate in part (Alternative C).

6. APHIS' analysis of data on agronomic performance, disease and insect susceptibility (to other insects other than the target insects), and compositional profiles of MIR604 and its non-genetically engineered counterpart indicates no significant differences between the two that would be expected to cause either a direct or indirect plant pest effect on raw or processed plant commodities from deregulation of MIR604. Based on this analysis, there would be no indirect or indirect plant pest effects on the raw or processed plant commodity by deregulating this line in whole (Alternative B). There would also be no impact from continuing to regulate the line (Alternative A) or to deregulate in part (Alternative C).

7. APHIS has reviewed field performance data submitted by the petitioner, and these data indicate that the engineered plant is not different in any fitness characteristics from its parent that might cause MIR604 to become invasive.

8. None of the alternatives are expected to have significant human health or environmental effects.

Because APHIS has reached a finding of no significant impact, no Environmental Impact Statement will be prepared regarding this decision.

Cindy Smith
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: MAR 16 2007

Attachment
Finding of no significant impact
Response to comments
APHIS No. 04-362-01p

On January 10, 2007, APHIS published a notice in the Federal Register (72 FR 1212-1214, Docket no. 2006-0157) announcing the availability of the draft EA for public review and comment for a 30-day comment period, ending February 9, 2007, which was extended to close on March 9 (72 FR 7952), and the availability of the subject petition for public review and comment for a 60-day comment period, ending March 12, 2007. APHIS received 14 comments regarding the EA and 27 comments regarding the petition. APHIS' responses to the issues raised during the comment periods are included below.

APHIS reviews the petition to determine if the genetically engineered (GE) organism should continue to be considered a regulated article under the APHIS biotechnology regulations found at 7 CFR Part 340. In order for a GE organism to be considered a regulated article under these regulations, the organism must pose a plant pest risk and be modified by recombinant DNA techniques (genetic engineering under the definition of the regulation). Prior to making a decision on a petition for APHIS to grant nonregulated status for a GE organism, APHIS prepares an EA to evaluate the significance of impacts on the environment arising from a decision to grant nonregulated status. APHIS prepares the EA as part of its obligation, like other Federal agencies, to meet the requirements of the National Environmental Policy Act of 1969 (NEPA). As part of a petition, APHIS considers public comments on the proposed deregulation as well as the EA that APHIS prepares under NEPA.

The APHIS response to comments below has also been reflected in some revisions and clarifications of the initial draft EA, so that the revised, final EA takes these issues into account. The final EA, along with the finding of no significant impact (FONSI) and response to comments are made available to the public.

Commenters in support of the petition for nonregulated status stated that the use of MIR604 could result in reduced use of chemical insecticides needed to control the corn rootworms. This reduced use of chemical pesticides could, in turn, save growers the time and expense of chemical insecticide applications to control these pests. In addition, commenters in support of the petition stated that the reduced use of chemical pesticides should result in health benefits to humans and the environment in general. In particular, several comments mentioned the increased safety afforded by such genetically engineered corn varieties like MIR604 as compared to the use of chemical pesticides, citing that chemicals have been shown to be carcinogenic, cause mutations, and damage the environment. One comment mentioned the existence of scientific support for the effectiveness of plants engineered to contain Bt proteins to control insect pests of plants.

APHIS agrees that if MIR604 were to be used to control rootworm pests in corn, it would likely be safer for growers to handle than the chemical insecticides that are presently registered for the control of corn rootworm pests. EPA registration data on the respective chemical pesticides and the Biopesticide Registration Action Document (BRAD) for MIR604 support this conclusion, and this is a consideration that EPA has taken into account for the registration of the Bt protein

that is expressed in MIR604. For more information on the EPA registration of the plant-incorporated protein expressed in MIR604, see United States Environmental Protection Agency, "Biopesticides Registration Action Document: Modified Cry3A Protein and the Genetic Material Necessary for its Production (Via Elements of pZM26) in Event MIR604 Corn SYN-IR504-8," March, 2007, available at http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006509.pdf

APHIS recognizes that the envisioned environmental benefits of MIR604 will depend on how widely it is adopted by corn growers. It is not possible for APHIS to be able to predict whether corn growers will use MIR604 corn and varieties developed from it. Other Bt-corn varieties are currently available for cultivation by U.S. corn growers for the control of corn rootworm. For more information on the varieties currently available for U.S. growers interested in rootworm control, see the online database maintained by the National Corn Growers Association (http://www.ncga.com/biotechnology/Search_hybrids/know_where.asp). The database lists 32 seed companies that currently market Bt varieties for the control of corn rootworm.

Other commenters in support of the petition stated that control of corn rootworm has another advantage over chemical insecticides, because its effectiveness is not affected by soil moisture. This is unlike the chemical insecticides that require sufficient moisture to dissolve the insecticidal compound through the soil to the rootworms, yet not too much moisture such that the concentration of the insecticide is diluted beyond effective concentrations.

APHIS agrees that the potential for increased reliability of MIR604 for rootworm control, compared to chemical insecticides, might be advantageous to growers. Such an advantage may increase the likelihood of growers choosing Bt-mediated control of corn rootworm pests, but it is not possible for APHIS to predict whether growers would choose varieties derived from MIR604 rather than the currently available Bt-corn varieties that utilize a similar Cry3A protein as the plant-incorporated protectant to provide control of corn rootworm species.

Other commenters in support of the petition stated that MIR604 is likely to have increased yield (more grain produced per acre), and that this would help to use land more efficiently so that there is less need for increasing land used for corn production. In other comments in support of the petition stated that they envision that the increased yield of MIR604 are necessary due to the expanding uses of corn for ethanol production as well as domestic and international demands.

APHIS can not make conclusions on the future yields of MIR604 and varieties derived from it, because there is insufficient information. If MIR604 is granted nonregulated status from APHIS it will be possible for corn breeders to more readily develop a wide range of corn varieties to meet the needs of corn growers throughout the diverse areas where corn is grown in the U.S. The yield of these varieties will depend on many factors, and the production of the Cry3A protein is likely to be only one of many factors in evaluating overall yield of these varieties. The EPA registration of the mCry3A protein produced for rootworm control in MIR604, along with an APHIS determination of nonregulated status would likely be important factors to encourage corn breeders to develop varieties that incorporate both this resistance trait as well as traits to promote good yields, but there are many other factors that preclude APHIS from predicting whether such varieties will be developed by breeders and adopted by growers. APHIS concludes

that the adoption of MIR604 in U.S. agriculture would be only one of a myriad of factors involved in the future cultivation of corn for food, feed, and industrial uses, including any use in ethanol production.

Other commenters in support of the petition stated that the use of MIR604 in U.S. corn production can address increasing challenges in corn rootworm control, especially for those rootworms that exhibit extended diapause. Extended diapause results in the temporary suspension of insect development. Individual rootworms with extended diapause escape the controlling effects of insecticides and thereby lead to the development of rootworm populations that are resistant to the insecticide. Because the insecticidal mCry3A protein produced by MIR604 is slightly different from the Cry3A protein that has been engineered into the Bt corn varieties that are currently available for cultivation, the use of MIR604 varieties may be a useful way to reduce the likelihood that rootworms will develop resistance to Cry3A.

APHIS agrees that the use of MIR604 may be a useful strategy to slow the development of resistance in rootworm populations to the Cry3A expressed in the varieties currently available to U.S. corn growers. APHIS addressed this point in the draft EA. APHIS has discussed this issue in consultations with EPA during the course of the petition review and notes that the EPA registration for the mCry3A protein and the insect management take these issues into account. Such consultations between APHIS and EPA occur whenever APHIS is reviewing a petition for nonregulated status of a GE plant that is engineered to produce a plant-incorporated protectant. More information on the EPA's registration can be obtained in the EPA document cited above and at the conclusion of this section on response to comments.

Other commenters in support of the petition stated that another Bt-corn variety product would provide growers with additional choices when choosing among Bt-corn varieties for the control of corn rootworm. Commenters stated that such increased choice would create a more competitive marketplace, because there is currently only one supplier of such a corn variety.

APHIS agrees that the determination of nonregulated status of MIR604 may result in the availability of additional Bt-corn varieties for U.S. corn growers, but APHIS concludes that there are other market factors that are likely to play a large role in whether additional varieties based on MIR604 are developed and adopted in U.S. corn production. APHIS does not agree with the commenter's statement that there is only one supplier of a corn variety that has been engineered to produce a Cry3A protein for the control of corn rootworm. APHIS bases this conclusion on readily available information, including the database of the National Corn Growers Association (see citation to the online database above).

Several comments in support of the petition discussed the cost of chemical insecticides incorporated into the soil to control corn rootworm. One commenter added that the high cost of these chemical insecticides puts them at a competitive disadvantage compared to farmers who do not use these chemical pesticides.

APHIS agrees that the costs for the control of corn rootworm by chemical insecticides can be important factors when growers choose their approaches to controlling corn rootworm. Based upon the apparent adoption by growers of existing Bt-corn varieties for the control of corn

rootworm, APHIS concludes that such varieties have provided growers with additional options for control that are economically advantageous to growers. However, APHIS notes that there are insufficient formal studies to draw a complete picture of the economic advantages for growers to adopt MIR604-dervived varieties in the future. As a result, APHIS can not definitively corroborate or refute the claim of this commenter.

Another commenter in favor of the petition was the petitioner, Syngenta. The petitioner submitted several documents that support the statements made in its comment, including: (1) the U.S. EPA fact sheet on MIR604 corn entitled "MCry3A protein and the genetic material necessary for its production (via elements of pZM26) in event MIR604 corn SYN-IR604-8 (006509) Fact Sheet" which reiterates the conclusions contained within EPA's BRAD that "the Agency [EPA] has determined that the use of this pesticide is in the public interest and that it will not cause any unreasonable adverse effects on the environment during the time of conditional registration."; (2) a letter from FDA to Syngenta dated January 30, 2007, that states FDA's conclusion that 'based on the safety and nutritional assessment Syngenta has conducted, it is our understanding that Syngenta has concluded that corn grain and forage derived from the new variety are not materially different in composition, safety, and other relevant parameters from corn grain and forage currently on the market and that genetically engineered corn event MIR 604 does not raise issues that would require premarket review or approval by FDA ...we have no further questions concerning grain and forage derived from corn event MIR604 at this time."; (3) a copy of the Agrisure stewardship agreement that details grower responsibilities if they purchase and grow seeds of MIR604 corn; and (4) a copy of a guide for MIR604 corn growers, including details on EPA-mandated IRM strategy, grain marketing with biotech corn traits, details on pollen movement and identity preservation production, and weed resistance management.

APHIS concludes that these documents provide additional support of the proposed APHIS decision to grant nonregulated status in whole to MIR604 corn. These documents support the conclusion that APHIS reached in its own review that a decision to grant nonregulated status to MIR604 should not pose a significant impact to the environment.

Comments in opposition to the petition were received from three individual citizens and from two organizations. The comments from the individual citizens expressed general disapproval of all genetically engineered organisms, and that genetically engineered organisms should not be allowed in food or feed.

APHIS recognizes that some citizens are opposed to the notion of genetically engineering organisms in general. Federal, state, and local governments have recognized this view since the techniques for copying genes from one organism and moving them to another were first developed over 36 years ago. The safe use of the techniques of genetic engineering has been at the heart of Federal efforts to ensure the safe use of genetic engineering. Toward this goal at the Federal level, the Office of Science and Technology Policy published the final version of the Coordinated Framework for the Regulation of Biotechnology in 1986 after considering extensive public comments received on the draft document that was made available in late 1984. The Coordinated Framework of 1986 set out the national policy to provide for the safe use of genetic engineering in the U.S. The respective regulatory responsibilities and relevant laws were

described in the framework, including the roles for food safety under the jurisdiction of the Food and Drug Administration (FDA), pesticide safety under the jurisdiction of the Environmental Protection Agency (EPA), and environmental protection under the USDA. As part of the Coordinated Framework, the relevant agencies are intended to coordinate their efforts under their respective statutory authorities. As part of the USDA, APHIS coordinates closely with FDA and EPA when it receives a petition to grant nonregulated status, yet APHIS statutory authority is separate from that of FDA and EPA. More information on the Coordinated Framework and the respective roles of the FDA, EPA and USDA is provided online at the U.S. Regulatory Agencies Unified Website (http://usbiotechreg.nbii.gov/).

Under the Coordinated Framework, the APHIS role is to protect against risks to plants, i.e., direct or indirect harm. In the case of MIR604, APHIS evaluates the plant to decide if it is likely to pose a plant pest risk. Then APHIS prepares an EA to evaluate the significance of any environmental impacts that are likely to arise from a decision to grant nonregulated status. The safe use of MIR604 corn as food or feed is under the jurisdiction of the FDA. The safe use of the plant-incorporated protectant in MIR604, the mCry3A protein, is under the jurisdiction of the EPA. The EPA also establishes the levels of pesticides that can safely be present on food and feed.

APHIS concludes that the relevant regulatory reviews under the Coordinated Framework provide for the safe use of MIR604 and the protection of the environment, including humans.

Another comment in opposition to the petition stated that it was unsafe to eat genetically engineered plants and cited a study conducted by Monsanto that the commenter believed supported the conclusion that genetically engineered plants were unsafe to rats and other animals.

APHIS does not agree with the conclusion that the cited study demonstrated that genetically engineered plants were unsafe to eat. In addition, the study cited by the commenter did not even test the effects of MIR604 or the mCry3A protein expressed by MIR604. The MIR604 corn has successfully completed the consultation process with FDA for food and feed safety, and EPA has proposed that the mCry3A is exempt from a tolerance because of the safety as food and feed of the mCry3A protein and the genetic elements necessary for its producing this protein.

Comments in opposition to the petition were received from three organizations: two grain trade associations and an environmental advocacy group. The two grain trade associations opposed the petition, because they believe that Syngenta is marketing MIR604 in advance of being granted nonregulated status by APHIS and regulatory approval in foreign countries.

APHIS biotechnology regulations are pursuant to the Plant Protection Act (PPA), which is a safety statute intended to protect plant health in the U.S. As long as MIR604 is a regulated article under APHIS regulations (7 CFR Part 340), it is subject to the provisions of the regulation under the PPA, which is not a marketing statute. Although APHIS engages in technical dialogs with its regulatory counterparts in other countries, the decisions in the other countries are sovereign decisions of those countries. Likewise, APHIS decisions are made under its

regulations and the PPA. Any future marketability of MIR604 in countries outside the U.S. is the responsibility of those who wish to market it in those countries.

The U.S. corn growers have developed a cooperative and coordinated approach to enable growers to choose corn varieties that takes into account the regulatory approvals that have been obtained in each of the countries where corn is marketed. Details on the mechanism and its broad applicability can be seen in the online information shared by the National Corn Growers Association, including the database link (http://www.ncga.com/biotechnology/Search_hybrids/know_where.asp). In reviewing this information, and in discussions with corn growers in the U.S. it is clear to APHIS that corn growers have developed a workable system to allow growers to preserve marketability both in the U.S. and abroad for GE corn varieties that have received cleared regulatory reviews in the U.S., but not in all other countries that are markets for U.S. corn production. In addition, Syngenta provided a comment that they intend to sell MIR604 corn to growers with a requirement that the grower not market it for export.

In a further comment in opposition to the petition, the grain trade associations called for Syngenta to provide testing methods to detect MIR604 in the grain that might be produced by U.S. growers if APHIS grants nonregulated status to MIR604.

APHIS concludes that this request from the grain trade associations is based on the marketability of MIR604, rather than its safety. As described above, the APHIS legal authority is for safety, not marketability. APHIS concludes that there are sufficient mechanisms already being used by U.S. corn growers and marketers to address this issue.

In a further comment in opposition to the petition, the grain trade associations called for seeds of MIR604 to be clearly labeled so that growers will be able to determine if this corn has the requisite regulatory approvals in certain countries that may be markets for U.S. corn.

APHIS concludes that if MIR604 seed is sold in the U.S. it will be clearly labeled because of the registration requirements under EPA's authority. The EPA already requires clear labeling of plant-incorporated protectants (such as the mCry3A protein in MIR604) and other pesticides. This is a well established practice that has been in place for many years, and is currently being used for many varieties of corn which express plant-incorporated protectants.

Comments in opposition to the petition were received from an environmental advocacy group that identified a number of concerns related to the petition itself and the draft EA prepared by APHIS. Overall, this group expressed concern that APHIS did not adequately consider the results of a scientific advisory panel (SAP) convened by the Environmental Protection Agency (EPA) on March 14-15, 2006 (1). The environmental advocacy group cited several examples, and these are addressed in turn below:

The environmental advocacy group claimed that APHIS failed to appropriately consider the SAP's views and studies referenced in the SAP, that allegedly demonstrated that "Several studies of other types of Bt corn have indicated that the insecticidal Bt toxin (e.g. Cry1Ab) leaks from

the roots of the corn, persists in the soil by binding to clay particles in the soil, and that Bt corn residues decompose more slowly than non-Bt corn residues."

APHIS rejects this assertion on several grounds. First, because the actual purpose of the study was "to test the inherent degradability of mCry3A in soil typical of corn-growing areas and with healthy microbial activity". The study actually concluded that the study noted the lack of persistence or accumulation of the Cry proteins in soil was a result of degradation by protein-degrading enzymes that are usually common in soil. They concluded that these results were consistent with other published scientific studies which had also demonstrated the lack of accumulation or persistence of Cry proteins in soils where other Bt crops have been grown.

The environmental advocacy group further criticized the APHIS analysis by citing criticisms that were raised by some members of the EPA's SAP regarding whether the mCry3A protein produced in bacteria should serve as an appropriate substitute for mCry3A protein produced in corn.

APHIS rejects this criticism. The production of test Cry proteins in bacteria is a common and widely accepted approach in evaluating certain characteristics of these proteins. It is used commonly, in part, because the protein is produced in relatively low concentrations in engineered plants, whereas large amounts of the protein can be produced in engineered bacteria. This approach is widely accepted and considered valid if the resulting protein has similar biochemical and biological properties to the protein produced in the engineered plant. This was concluded by comparing the molecular weight, amino acid sequence, lack of glycosylation of the protein, as well as the biological activity against the target coleopteran species, Western corn rootworm (WCRW). APHIS and EPA reviewers reached the same conclusion independently that the protein produced in bacteria would serve as an appropriate test protein to evaluate the mCry3A produced in MIR604.

The environmental advocacy group claimed that APHIS failed to appropriately consider the SAP's views summarizes its comments regarding APHIS apparently ignoring "the most relevant and well-credentialed independent assessment of Syngenta's data."

APHIS did take into account not only the SAP report, but considered the report in the context of the issues it addressed, the diversity of viewpoints expressed among the members of the SAP, and the overall analysis and review that are summarized in the EPA decision to grant registration (U.S. EPA SAP, 2006). The BRAD was not yet published at the time that APHIS published its draft EA. It is clear in the BRAD that EPA recently published that EPA has clarified the diverse comments of the SAP, puts them into context, and concludes that "the Agency has determined that the use of this pesticide is in the public interest and that it will not cause any unreasonable adverse effects on the environment during the time of conditional registration" (U.S. EPA BRAD, 2007).

APHIS did take the results of the EPA's SAP into consideration, along with the totality of reviews undertaken by EPA. EPA often uses its authority under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), to convene SAPs to consider specific technical questions related to the proposed registration of a pesticide. EPA chooses members of the SAP

for their technical expertise on the specific issues under consideration, then considers the SAP conclusions and recommendations in the totality of all information that EPA considers before making a decision on registration of a pesticide. EPA convened a SAP to address scientific issues that arose during the risk assessment of mCRY3A, the insecticidal protein engineered to be expressed in MIR604 corn. The EPA reviewed the conclusions from the SAP, along with the other information reviewed by EPA, in order to determined if it could issue a finding that the product "will perform its intended function without unreasonable adverse effects on the environment." 7 U.S.C. § 136(c)(5). On March 9, 2007, EPA electronically posted its decision in the "Biopesticides Registration Action Document: MCry3A Protein and the Genetic Material Necessary for its Production (Via Elements of pZM26) in Event MIR604 Corn SYN-IR604-8" (http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006509.pdf). This document reflects the totality of the EPA review, including those issues addressed when EPA sought the additional input from the SAP.

APHIS has considered the BRAD and the EPA review in the preparation of the final EA and in its response to comments. APHIS agrees with the EPA conclusion that the plant-incorporated protectant under review "will perform its intended function without unreasonable adverse effects on the environment."

In another comment in opposition to the petition from this same environmental advocacy group, they criticized APHIS for the apparent conflict in approach between APHIS and EPA. The environmental group complains that an APHIS determination of nonregulated status for MIR604 is "permanent", even though the EPA registration for the plant-incorporated protectant in MIR604 calls for the registrant to submit additional studies to EPA for each year of the first three years of the registration (studies to focus on protein accumulation, degradation, and persistence is a variety of soil types (see full text of US-EPA BRAD in citation below).

APHIS disagrees with this claim by the commenter that APHIS and EPA regulatory decisions are in conflict. EPA's BRAD clearly concludes that the plant-incorporated protectant in MIR604 will perform its intended function without unreasonable adverse effects on the environment. It is not uncommon for EPA to request additional information from registrants, and it is common practice for registrants to continue to gather data on pesticide performance and environmental impacts and benefits prior to periodic re-registration of pesticides. For its part, APHIS has concluded that there is sufficient evidence to support its conclusion of no significant impact to the environment from a decision to grant nonregulated status to MIR604. APHIS would like to respond to the commenter's notion of "permanent" nonregulated status. APHIS has broad authority under the PPA and its regulations to protect plant health, and this includes re-regulation if needed of any genetically engineered organism that the Administrator has reason to believe is posing a plant pest risk (including any organism that had previously been granted nonregulated status).

In another comment in opposition to the petition from this same environmental advocacy group, they claim that the U.S. regulatory system does not provide adequate oversight over genetically engineered crops.

APHIS rejects this claim on several grounds. First, this criticism of the US regulatory system is

directed at those genetically engineered plants that APHIS regulates, rather than the issue at hand with this petition – namely granting nonregulated status to a genetically engineered plant that APHIS has concluded poses no greater plant pest risk than any other variety of corn that is being grown without APHIS regulation. Second, APHIS concludes that the evidence to date supports the conclusion that the U.S. regulatory system has been effective in providing safety and allowing researchers to develop innovative, improved plant varieties. Since 1987, BRS has carried out an effective regulatory program for GE plants that has provided both safety and the opportunity for plant breeders to develop improved varieties to meet growers' needs. During this 20 year period, APHIS has developed and continued to refine a risk-based regulatory approach in implementing the APHIS notification and permitting procedures used when GE plants are imported, moved interstate, and released into the environment for confined field tests. These field tests make it possible to evaluate the performance of the GE plants under conditions that are similar to the ways they might eventually be used in agriculture. This regulatory approach has worked well, and to date APHIS has effectively overseen approximately 12,000 field tests under its notification procedure and another 1,500 field tests under the permitting procedure. This regulatory approach has a proven track record of effectiveness. APHIS has worked closely and cooperatively with its regulatory counterparts in EPA and FDA in developing and implementing each agency's role under the Coordinated Framework for Regulation.

In another comment in opposition to the petition from this same environmental advocacy group, they call for a moratorium on commercialization of genetically engineered plants.

APHIS rejects this proposal for a moratorium on several grounds. Such an approach would contradict the national policy as described in the Coordinated Framework for Regulation, which states that the mere fact of using genetically engineering to modify an organism does not mean that the organism necessarily poses a greater risk. Rather, the regulatory approach focuses on the characteristics of the organism or product, and how the organism or product is to be used.

In another comment in opposition to the petition from this same environmental advocacy group, they claimed that APHIS inappropriately abdicated its authority to FDA and EPA. Specifically, they state that the APHIS draft EA failed to adequately discuss the environmental impacts of increased pesticide residues associated with Bt corn.

APHIS disagrees with this assertion. The APHIS draft EA included information summarizing analysis of insecticide use necessary for control of corn rootworms, as well as detailed information on chemicals currently used for corn rootworm control (Appendix B). APHIS also stated that as part of their analysis they considered a published study that concluded that the currently available Bt seed technologies for corn have resulted in the reduction of chemical insecticides. In the final EA, APHIS provides additional information on nontarget impacts that provides further support for its conclusion that the cultivation of MIR604 is unlikely to pose significant impacts on nontarget species. In aggregate, APHIS concludes that the cultivation of MIR604 for rootworm control is likely to have fewer environmental impacts than current control methods that rely on chemical insecticides. This conclusion is further supported by a review of the effects on nontarget organism that are presented by the chemical insecticides as compared with Cry3A and mCry3A. Because of this fact, the residues of mCry3A are likely to be safer for

nontarget organisms than the chemical insecticides currently registered for control of corn rootworm species.

In another comment in opposition to the petition from this same environmental advocacy group, they claimed that APHIS failed to address food related issues such as allergenicity and human health impacts.

APHIS disagrees with this assertion. In the draft EA, APHIS concluded that petition data, as well as information in the scientific literature, were sufficient to demonstrate that MIR604 is unlikely to impact human health. APHIS concluded that toxicity and allergenicity data in the petition were consistent with the information in the published scientific literature. In aggregate, these data supported a conclusion that the mCry3A protein and the PMI enzyme are unlikely to pose a threat to human health. In addition, Appendix B of the draft EA summarized results of a feeding study involving mice that supported the APHIS conclusion.
The EPA review also accepted the acute oral mouse study submitted by Syngenta as supporting evidence that led EPA to conclude that "the potential for the mCry3A protein to be a food allergen is minimal" in their final registration for MIR604. APHIS and EPA reviews each took into account numerous characteristics of the mCry3A protein, including (1) the amino acid sequence of mCry3A has no significant similarity with proteins known to be or suspected to be allergens; (2) an in vitro digestibility study on mCry3A demonstrated that this protein is rapidly digested; (3) the mCry3A protein originates from an organism not known to be a source of allergens; and (4) the mCryA protein is not glycosylated when expressed in corn. EPA concluded that the weight of evidence was sufficient to respond to questions raised by some members of their SAP. In addition to this evidence on mCry3A, there is the additional evidence from the use of other Cry proteins that they are not allergenic (a variety of Cry proteins serve as the active ingredient in microbial Bt biopesticides as well as plant-incorporated protectants). At the time that APHIS published the draft EA, the FDA had not yet completed the food safety consultation on MIR604, but that consultation was successfully completed on January 30, 2007. Under the Coordinated Framework, the food related issues such as allergenicity are addressed by the other agencies. In the case of MIR604, the reviews and conclusions by the other agencies have been taken into account (in general, allergenicity is part of the FDA review process; EPA reviews for allergencity in the case of plant-incorporated protectants). Considering its own reviews and the reviews of EPA and FDA, APHIS concludes that there is not a significant risk to human health by granting nonregulated status to MIR604.

In another comment in opposition to the petition from this same environmental advocacy group, they also criticized FDA's consultation process.

While this comment is specific to FDA, APHIS consults frequently with FDA and EPA in the course of the APHIS review of petitions for nonregulated status. APHIS has first-hand knowledge of the scientific rigor that each of the agencies of the Coordinated Framework bring to bear when conducting reviews. This knowledge provides APHIS with additional confidence in its own conclusion that a determination of nonregulated status for MIR604 should pose no significant impact on the environment.

In another comment in opposition to the petition from this same environmental advocacy group, they claimed that the APHIS draft EA was inadequate due to its acceptance of Syngenta's dietary toxicity studies.

APHIS disagrees with this assertion, because Syngenta studies were only part of the information that APHIS used to reach its conclusion that MIR604 is unlikely to be toxic to organisms other than certain coleopteran insect species. There is corroborating evidence in the scientific literature on similar Cry3A proteins that have been widely studied for over 20 years. The APHIS conclusion of safety has been supported by the independent review that EPA has made in the course of their registration of mCry3A.

In another comment in opposition to the petition from this same environmental advocacy group, they claimed that APHIS should have required the petitioner to submit mCry3A toxicity tests on marine and estuarine organisms.

APHIS has concluded that such data were not necessary, because there is enough information in the scientific literature to support the conclusion that neither the PMI nor the mCry3A protein like likely to be toxic to organisms in organisms in marine and estuarine habitats. Only mCry3A exhibits any toxicity, and then it is restricted to some insects in the order Coleoptera (this order includes beetles). Even if beetle larvae were in marine or estuarine habitats and they were sensitive to mCry3A, it is unlikely that they would be exposed to mCry3A. APHIS reached this conclusion in light of the fact that corn is not grown in these habitats and any corn debris reaching these habitats would be extremely dilute and subject to degradation and digestion by a wide range of macro- and micro-organisms.
Risk is influenced by both the nature of the hazard and the exposure, and in this case the APHIS analysis has concluded that both hazard (toxicity) and exposure are negligible.

In its draft EA, APHIS noted that many of the endangered and threatened beetles occur in caves and aquatic habitats and not in or near cornfields, except for the American burying beetle, which was not expected to occur within cornfields and therefore would not likely be exposed to mCry3A.

In another comment in opposition to the petition from this same environmental advocacy group, they claimed that APHIS did not adequately address potential impact to the endangered beetle species, Hungerford's crawling water beetle, which is found in corn production areas of Michigan's northern Lower Peninsula.

APHIS has addressed this issue more thoroughly and references the U.S. Fish and Wildlife Service's fact sheet on this species (http://www.fws.gov/Midwest/endangered/insects/hungerfo.html) which states that this beetle is thought to feed on algae that grows on rocks or stones in streams. This feeding behavior and preference for areas of moderate to fast flow and good aeration indicate that the likelihood of exposure to mCry3A proteins would be extremely unlikely. In the event that MIR604 plant tissue or mCry3A proteins from cornfield soils entered into these riparian environments, it would be quickly dissipated, and exposure would be negligible.

Another reason why APHIS has concluded that there is low risk to the Hungerford's crawling water beetle is because the mCry3A is toxic only to insects of a specific suborder of Coleoptera, namely Polyphaga. This suborder includes the corn rootworm, but not other coleopteran species such as Hungerford's crawling water beetle and *Poecilus cupreus*, (these species are in the suborder Adephaga). The expected insensitivity of *Poecilus cupreus* was confirmed in data submitted by the petitioner. In these toxicity assays, *P. cupreus* was not sensitive to the mCry3A protein, even when exposed to unrealistically high levels (11.2 times the expected environmental concentration). These results further support the APHIS conclusion that MIR604 does not pose any 'unreasonable adverse effects…to endangered coleoptera.' APHIS concludes that there will be no significant environmental impact to federally-listed threatened or endangered species from the granting of nonregulated status to MIR604 corn.

In another comment in opposition to the petition from this same environmental advocacy group, APHIS is criticized for relying on data from a single study conducted by the petitioner to evaluate the degradation of mCry3A in soil, specifically Syngenta's use of a single study site, the use of a bacterial surrogate mCry3A protein rather than MIR604 crop residues, and the amount of mCry3A earthworms that were exposed to in the toxicity study.

APHIS disagrees with the criticism that its analysis did not have adequate information to reliably reach the conclusion that MIR604 crop residues were unlikely to pose an impact on earthworms. APHIS considered the petitioner's study along with information in the scientific literature regarding the effect of other Cry3A proteins on nontarget species, including earthworms. The study results submitted in the petition corroborated previous studies with other Cry3A proteins.

In another comment in opposition to the petition from this same environmental advocacy group, they claimed that the APHIS draft EA did not adequately analyze cumulative impacts of the proposed decision to grant nonregulated status.

APHIS agrees that its draft EA did not describe cumulative impacts in great detail. The draft EA did address some potential cumulative impacts within the section in the EA on agricultural and cultivation practices, stating that "the availability of this product is likely to have an impact on current control practices for corn rootworm that include the use of crop rotation, chemical insecticides, and other Bt corn varieties that are intended to control corn rootworm." APHIS noted that some corn rootworm populations have developed extended diapause which also serves as a mechanism of resistance, to a current CRW control strategy of crop rotation.

The final EA describes the APHIS analysis in greater detail, and some additional information is presented here in the response to this comment. As described above, it is somewhat speculative for APHIS to predict to what extent MIR604 corn might be used by U.S. corn growers, assuming a determination of nonregulated status from APHIS. The prevalence of this and other Bt corn varieties in actual production depends on many factors, including pest prevalence and severity, the availability of other control measures, as well as the economic costs and benefits to growers. Even the price that growers receive for corn grain will influence this complex calculus. Considering the multiplicity of factors, their magnitudes, and their interactions, it is beyond the ability of APHIS or anyone to reliably predict. All of these complex factors notwithstanding, APHIS has considered that a range of possible cumulative impacts that might arise as a

consequence of granting nonregulated status to MIR604 corn. Some of the cumulative impacts might arise from decreased reliance on chemical pesticides for corn rootworm control. If varieties based on MIR604 merely replace some existing Bt corn varieties that are resistant to rootworms, there may be little or no net effect. Because the mCry3A protein of MIR604 differs slightly from the Cry3A protein in the corn varieties currently available for growers in the U.S., the use of MIR604 could possibly delay the development of rootworm populations resistant to either or both types of Cry3A. Further discussion of potential cumulative impacts can be found in the final EA that APHIS publishes with this response to comments.

In another comment in opposition to the petition from this same environmental advocacy group, they claimed that MIR604 might lead to the development of antibiotic resistance to "clinically critical antibiotics."

APHIS does not agree with this assertion, because MIR604 was not engineered with antibiotic resistance gene. APHIS believes the commenter did not realize this when the comment was submitted. To create MIR604, the petitioner engineered two genes into corn, specifically the mCry3A and PMI genes.

In another comment in opposition to the petition from this same environmental advocacy group, they claimed that the use of MIR604 corn in U.S. agriculture will result in the development of resistance in corn rootworm populations to mCry3A and similar Cry3-type proteins that are expressed by corn varieties currently grown by U.S. corn growers.

APHIS does not agree that the cultivation of MIR604 corn will necessarily result in the development of resistant rootworm populations. EPA has considered this issue in its review and recommendation for insect resistance management (IRM) strategies to be used by growers of MIR604 and other Bt corn varieties expressing plant incorporated protectants for the control of corn rootworm species. Because the Bt-based insecticides are generally more environmentally benign than most chemical insecticides, EPA has incorporated the use IRM strategies to prolong the useful life of these biopesticides. The APHIS draft EA noted that Bt-corn varieties resistant to rootworm have been grown since 2002, and resistance in rootworm populations has not developed.

APHIS also notes two typographical errors in the section '*Potential impacts on threatened and endangered arthropods*' where there is a spelling error and an omitted word within the fifth sentence. In the revised, final EA, the noted section now correctly reads "APHIS has thoroughly examined all threatened and endangered coleopterans that occur in counties where corn is grown, and determined that the breeding habitat of **coleopterans** does **not** put them in proximity of corn fields.'

Supporting documents

United States Environmental Protection Agency, "A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding Event MIR604 Modifie5d Cry3A Protein Bt Corn – Plant Incorporated Protectant," Minutes of a Meeting of the FIFRA Scientific Advisory

Panel, held March 14-15, 2006, June 1, 2006, available at http://www.epa.gov/scipoly/sap/meetings/2006/march/finalmeetingminutes6_1_2006.pdf,

United States Environmental Protection Agency, "Biopesticides Registration Action Document: Modified Cry3A Protein and the Genetic Material Necessary for its Production (Via Elements of pZM26) in Event MIR604 Corn SYN-IR504-8," March, 2007, available at http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006509.pdf

Public comments received in response to this docket can be viewed online at www.regulation.gov by entering the docket number in the search field.

Animal and Plant Health Inspection Service
Petition for Non-regulated Status for Corn Line MIR604 (APHIS 04-362-01p)

Additional APHIS response to comments received on petition

In the response to public comments, APHIS mistakenly characterized the comments received from the two grain trade associations as being opposed to the petition. They contacted APHIS to clarify that they were not opposed to the petition, although they did have serious concerns about Syngenta's announced plans to commercially market seeds containing MIR604 corn prior to the relevant authorizations being obtained in countries that import U.S. corn grain. Their comment stated their belief that doing so could prove to be highly disruptive to international markets, and prove to be very costly to farmers, grain handlers and exporters.

These commenters also stated that in the past U.S. farmers and commercial buyers have relied upon technology providers acting responsibly to maintain access to major export markets by not allowing commercialization to proceed until major export markets also provided regulatory clearance for new biotechnology-enhanced events. The commenters stated that if Syngenta does market significant quantities of this seed prior to having regulatory approval in a country like Japan, it may well lead to widespread testing of corn lots potentially destined for export channels. They also stated that such an eventuality would, under existing technology, be totally impractical and/or highly costly, as the only known test that is reliable is a polymerase chain reaction (PCR) test.

It was for these reasons that the grain trade associations requested APHIS to seriously consider, if it chooses to deregulate the event, to impose the certain requirements related to the post deregulation commercialization of MIR604.

APHIS understands the position stated by the grain trade associations, but concluded that the three requirements and conditions proposed by the grain trade associations are focused on the mitigation of potential trade implications whereas the decision APHIS makes is focused on the safety of MIR604 corn. Once APHIS grants nonregulated status under its regulation, APHIS no longer has authority to compel the petitioner or anyone else to carry out the types of requirements and conditions proposed by these commenters. Nonregulated status means that the genetically engineered organism is not subject to the regulation at 7 CFR Part 340. The commenters have proposed that APHIS continue to place requirements on Syngenta and growers who use MIR604, even after APHIS grants nonregulated status to MIR604.

In the response to comments, APHIS cited the information shared by the National Corn Growers Association under their "Know Before You Grow" program as a way to provide some information to growers about the corn varieties on the market that are derived from genetically engineered corn lines. APHIS understands there is concern about the usefulness of this program when corn export markets have requirements in place that place a zero tolerance for the presence of any genetically engineered corn varieties that

have not yet received regulatory approvals in those countries. In such cases, even the slightest trace of an unapproved variety can result in rejection of the corn grain shipment. It was pointed out by the grain trade associations that it is not practical for a channeling system relying on such information to allow growers to preserve marketability both in the U.S. and abroad for GE corn varieties that have received cleared regulatory reviews under some conditions.

APHIS recognizes that a determination of nonregulated status for MIR604 may present serious marketing challenges for producers, the grain trade and export customers, but APHIS concludes that these challenges are not the result of the risks to the environment or plant health posed when APHIS grants nonregulated status.

USDA-APHIS
Final Environmental Assessment

In response to petition application (04-362-01p), received from Syngenta Seeds, Inc., for determination of nonregulated status of a corn variety (MIR604) genetically engineered to be insect resistant

U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services

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I. Summary

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared this final Environmental Assessment (EA) in response to a petition (APHIS Number 04-362-01p) from Syngenta Seeds, Inc. (Syngenta) regarding the regulatory status of genetically engineered (transformed) corn rootworm resistant corn derived from transformation event MIR604. This corn is currently a regulated article under USDA regulations at 7 CFR Part 340, and as such, interstate movements, importations, and field tests of MIR604 corn have been conducted under permits issued or notifications acknowledged by APHIS. Syngenta petitioned APHIS requesting a determination that MIR604 corn does not present a plant pest risk, and therefore MIR604 corn and its progeny derived from crosses with other nonregulated corn should no longer be regulated articles under these APHIS regulations.

II. Introduction

Syngenta has submitted a "Petition for Determination of Non-regulated Status" to the USDA/APHIS (APHIS number 04-362-01p) for genetically engineered corn plants that are resistant to the feeding damage caused by: the northern corn rootworm (NCRW, *Diabrotica longicornis barberi* Smith and Lawrence); the western corn rootworm (WCRW, *D. virgifera virgifera* Le Conte); and the Mexican corn rootworm (MCRW, *D. virgifera zeae* Krysan and Smith). The corn rootworm (CRW) larvae damage corn by feeding on the roots of corn plants, thereby inhibiting the ability of the plant to absorb water and nutrients from the soil (Reidell, 1990). This leads to harvesting difficulties due to lodging of the weakened plants (Spike and Tollefson, 1991). Annual losses to growers because of CRW have been estimated to approach a billion dollars when taking into account both the costs of chemical controls and crop losses from CRW (USDA-ARS, 2003).

Bacillus thuringiensis (Bt) bacteria produce a group of related toxins (delta-endotoxins) that when ingested by susceptible insects (e.g., insects of the orders Coleoptera, Lepidoptera, Diptera) result in insect death. Preparations of Bt-containing delta-endotoxins have been used for decades as foliarly-applied biopesticides. However, these foliar applications are not routinely effective against CRW pests because the insect pests reside in the soil. Similar problems can be encountered with other, non-systemic, foliarly-applied chemical insecticides. The development and approval of transgenic corn plants expressing Bt delta-endotoxins active against coleopterans (e.g., modified Cry3A) should provide growers with another safe and efficacious option for the control of CRW.

Syngenta used recombinant DNA techniques to produce and introduce into corn, a restriction fragment containing the two transgenes: (1) the modified *cry3A* (*mcry3A*) gene encoding the mCry3A insect control protein and (2) the *pmi* (*manA*) gene from *Escherichia coli*, which encodes the enzyme phosphomannose isomerase (PMI) as a selectable marker. Expression of the

mcry3A gene by corn plants renders the corn line resistant to CRW. Regulatory elements for the *mcry3A* and *pmi* genes were derived from maize and *Agrobacterium tumefaciens*. These regulatory sequences are not transcribed and do not encode proteins. In addition to transgenes necessary for insertion into the plant genome, the T-DNA vector also contained within the backbone two genes: (1) Streptomycin adenylyltransferase, *aadA,* gene from *E. coli*, conferring bacterial resistance to the antibiotics erythromycin, streptomycin, and spectinomycin and (2) consensus sequence for the origin of replication and partitioning region from plasmid pVS1 of *Pseudomonas*. The DNA was introduced into corn cells using *Agrobacterium*-mediated transformation methodology with the T-DNA transformation vector designated pZM26. Syngenta's petition describes the genetic construction of MIR604 and presents scientific evidence that demonstrates that the final product does not contain any of the backbone sequences from the transformation vector, pZM26. Plant cells containing the introduced DNA were then selected by culturing in the presence of mannose. After the initial incubation with *Agrobacterium*, the broad-spectrum antibiotic cefotaxime was included in the culture medium to kill any remaining *Agrobacterium*. Because the transformed cells contain some sequences from a plant pest, they are explicitly subject to regulation under 7 CFR Part 340.

MIR604 corn has been field tested in the United States since 2001 as authorized by USDA notifications and permits listed in Table 1, on page 29 of the final revised petition. The list compiles a number of test sites in diverse regions of the U.S. including the major corn growing areas of the Midwest and winter nurseries in Hawaii and Puerto Rico. Field tests conducted under APHIS oversight allow for evaluation in a natural agricultural setting while imposing measures to minimize the risk of persistence in the environment after the completion of the test. Data are gathered on multiple parameters and are used by the applicants to evaluate agronomic characteristics and product performance and are used by APHIS to determine if the new variety poses a plant pest risk.

A. USDA Regulatory Authority

APHIS regulations at 7 CFR part 340, which are promulgated pursuant to authority granted by the Plant Protection Act (7 U.S.C. 7701-7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. This corn has been considered a regulated article because it was genetically engineered with regulatory sequences derived from a bacterial plant pest.

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

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

MIR604 corn is also subject to regulation by other agencies. The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including herbicides, be registered before distribution or sale, unless exempted by EPA regulation. Before a product may be registered as a pesticide under FIFRA, it must be shown that when used in accordance with widespread and commonly recognized practices, it will not cause unreasonable adverse effects on the environment.

Under the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 301 *et seq.*), pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Residue tolerances for pesticides are established by EPA under the FFDCA. The FDA enforces the tolerances set by the EPA. An exemption from the requirement of tolerance has been established for the PMI protein in all crops (69 *FR* 26770-26775). On October 27, 2004, the EPA announced two applications submitted by Syngenta: 1) a petition requesting an exemption from the requirement of a tolerance for residues of the mCRY3A protein and the genetic material necessary for their production in corn (69 FR 62688-62692) and 2) an application to register a pesticide product containing a new active ingredient (69 FR 62678-62680). On April 6, 2005, a temporary tolerance exemption was granted, exempting the requirement of a tolerance for residues of the mCRY3A protein and the genetic material necessary for their production in corn based on the conclusion that there was a reasonable certainty of no harm from consumption of the protein, as it is digestible in gastric fluid and not considered an allergen (70 *FR* 17323-17327). This temporary exemption was subsequently renewed (69 *FR* 11431-11433) and was set to expire on October 15, 2007 (71 *FR* 13269-13274). On January 25, 2006, the EPA announced the receipt of an application filed by Syngenta to amend an application for an Experimental Use Permit (EUP) to include the plant-incorporated protectant Event MIR604 mCry3A corn (71 *FR* 4141-4142). Also, on January 25, 2006, EPA announced Syngenta applied for an extension to the tolerance exemption expiring on October 15, 2006 (69 *FR* 11431-11433). The EPA held a meeting on March 14 and 15, 2006, of the Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (FIFRA SAP) to consider and review human health and environmental issues associated with MIR604 Modified Cry3A Protein Bt Corn Plant Incorporated Protectant. EPA recently issued a "Biopesticides Registration Action Document: Modified Cry3A Protein and the Genetic Material Necessary for its Production (Via Elements of pZM26) in Event MIR604 Corn SYN-IR604-8" (http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006509.pdf). The final document, namely the Biopesticides Registration Action Document, contains EPA's conclusions regarding the scientific issues brought up by the SAP. With the publication of EPA's registration document, APHIS will use this finalized information to provide additional scientific support to its consideration of potential environmental impacts.

FDA's policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 *FR* 22984-23005. Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g.

labeling) are resolved prior to commercial distribution of a bioengineered food. Syngenta submitted a summary of their safety assessment on February 25, 2005, and additional information on March 21, 2006. The Syngenta assessment to the FDA indicated no changes in composition, safety or other relative parameters. FDA, which has primary regulatory authority over food and feed safety, completed their consultation on MIR604 on January 30, 2007, and concluded that it had "no further questions concerning grain and forage derived from corn event MIR604."

III. Purpose and Need

APHIS prepared this EA before making a determination on the status of MIR604 corn as regulated articles under APHIS regulations. The developer of this corn, Syngenta, submitted a petition to USDA-APHIS requesting that APHIS make a determination that this corn shall no longer be considered a regulated article under 7 CFR Part 340. Under regulations in 7 CFR Part 340, APHIS is required to make a determination on the petition for non-regulated status. This EA was prepared in compliance with the National Environmental Policy Act (NEPA) of 1969 as amended, (42 U.S.C. 4321 *et seq*.) and the pursuant implementing regulations (40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372).

IV. Alternatives

A. No Action: Continuation as a Regulated Article

Under the no action alternative, APHIS would come to a determination that MIR604 corn and its progeny should continue to be regulated under 7 CFR Part 340. Permits or acknowledgment of notifications from APHIS would still be required for their introduction. APHIS would choose this alternative if there was actual evidence that the regulated article posed a plant pest risk, or if there was insufficient evidence to demonstrate a lack of plant pest risk from the uncontained cultivation of MIR604 corn and its progeny.

B. Determination of Nonregulated Status

Under this alternative, MIR604 corn and its progeny would no longer be considered regulated articles under 7 CFR Part 340. Permits or notifications to APHIS would no longer be required for introductions in the United States and its territories of MIR604 corn or its progeny. A basis for this determination would be a finding that MIR604 is unlikely to pose a greater plant pest risk than the non-modified organism from which it was derived based on information submitted in the petition as stipulated in 7 CFR § 340.6(c) and other information that the Administrator believes to be relevant to a determination. Unrestricted cultivation of the lines would be permitted by APHIS. Such a determination, however, does not preclude any restriction on the cultivation of this corn that might be placed by other regulatory agencies also having authority.

C. Determination of Nonregulated Status, in Part

The regulations at 7 CFR 340.6(d)(3)(i) state that APHIS may approve the petition in whole or in part. There are at least two ways in which a petition might be approved in part:

Approval of some but not all lines requested in the petition. In some petitions, applicants request that nonregulated status be granted to lines derived from more that one independent

transformation event. In these cases, supporting data must be supplied for each line. APHIS could approve certain lines requested in the petition, but not others.

Approval of the petition with geographic restrictions. APHIS might determine that the regulated article poses no significant plant pest risk in certain geographic areas, but may pose a significant plant pest risk in others. In this case, APHIS may choose to approve the petition with a geographic limitation stipulating that the approved lines could only be grown in certain geographic areas based on the identification of site-specific plant pest risks.

D. Preferred Alternative

APHIS has chosen Alternative B as the preferred alternative. This is based on the lack of plant pest characteristics in the MIR604 corn.

V. Affected Environment

A. Corn

Zea mays L. subsp. *mays* is a member of the *Maydeae* tribe of the grass family, *Poaceae*. It is a monoecious perennial plant that requires human intervention for its seed dispersal and propagation. The species is open-pollinated through wind movement of pollen. Additional information on the biology of maize can be found within the Organisation for Economic Co-Operation and Development (OECD) consensus document, which can be accessed at: http://www.oecd.org/LongAbstract/0,2546,en_2649_34385_8328413_119829_1_1_37437,00.html. Maize is primarily grown in the warm temperate climates (Norman *et al.* 1995) such as the 'Corn Belt' in the midwest United States, which consists of Iowa, Indiana, Illinois and Ohio as well as parts of South Dakota, Nebraska, Kansas, Minnesota, Wisconsin, Michigan, Missouri and Kentucky. The expression of the mCRY3A and the PMI proteins in the MIR604 corn line are not expected to alter the range of corn cultivation within the United States.

B. Corn Rootworm

Corn rootworms are the most serious insect pests in field corn in the U.S., costing growers millions of dollars each year in terms of insecticide use and crop loss (USDA-ARS, 2003). Historically, crop rotation has provided effective protection from CRW damage. More recently, however, the effectiveness of crop rotation has become more limited because of several factors:

1. Many growers now prefer to grow corn continuously, as opposed to using crop rotation. Continuous corn production is a practice that necessitates higher inputs of chemical insecticides. The percentage of continuous corn acreage in the eastern and western Corn Belt states treated with insecticides ranges from 7%-100% (Gianessi *et al.*, 2002).

2. Crop rotation is not an effective management strategy for southern corn rootworm (SCRW) because it not only has a wide host range, but also because multiple generations can be produced in the same cornfield (Gianessi *et al.*, 2002). Larvae of SCRW can be found on the roots of corn, peanuts, alfalfa and cucurbits. There may be two to three generations of SCRW per year. Adults become active and lay eggs in

the soil in late spring. These eggs hatch after one week and the larvae feed on corn roots for two to four weeks before pupating. A new generation of adults can emerge in mid-summer (Gianessi *et al.*, 2002).

3. A new NCRW biotype has exhibited extended diapauses in which some eggs can survive through a non-corn rotation to attack corn in a subsequent season (Ostlie, 1987; Tollefson, 1988; Gray *et al.*, 1998; Gianessi *et al.*, 2002). In South Dakota, Minnesota, Iowa, and Nebraska, the new NCRW biotype can diapause for two winters which allows the eggs to bypass the rotated crop and hatch in time to feed on the next corn crop (Gianessi *et al.*, 2002).

4. A new biotype of WCRW has appeared in central Illinois, northern Indiana and parts of Michigan that can lay eggs in soybean fields, so that the eggs hatch in the following season coinciding with the corn rotation (Onstad and Joselyn, 1999; O'Neal *et al.*, 1999; Gianessi *et al.*, 2002). This strain has spread rapidly since it was first observed in 1993, and it is expected to continue to spread throughout the Corn Belt.

As a result of these factors and the very damaging nature of the pest, the CRW complex is the most significant corn pest in the U.S. in terms of the amount of organophosphate pesticide used for pest control. The most common chemical regime is the application of a granular insecticide at planting, either banded or in-furrow. In some cases sprays are applied for adult suppression. Widespread use of chemical insecticides has raised concerns for worker safety, water contamination, and other environmental risks. Appendix B is a table comparing some of the most commonly used chemicals with respect to environmental fate and toxicity.

VI. Potential Environmental Impacts

Potential impacts to be addressed in this EA are those that pertain to the use of MIR604 corn and its progeny in the absence of confinement.

A. Potential impacts from gene introgression from MIR604 corn into its sexually compatible relatives.

MIR604 corn is expected to cross fully with other cultivated corn varieties. This section addresses the potential impacts arising from gene introgression of MIR604 corn with other sexually compatible relative species. In assessing the risk of gene introgression from MIR604 corn into its sexually compatible relatives, APHIS considers two primary issues: 1) the potential for gene flow and introgression; 2) the potential impact of introgression.

APHIS evaluated the potential for gene introgression to occur from MIR604 corn to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Cultivated corn, or maize, *Zea mays* L. subsp. *mays*, is sexually compatible with other members of the genus *Zea*, and to a much lesser degree with members of the genus *Tripsacum*.

In general, gene flow from cultivated agricultural crops to domesticated, wild or weedy relatives has most likely occurred ever since the domestication of a particular crop, assuming sexually compatible species are present (Stewart *et al.* 2003). Based upon currently available data, there

have been a relatively low number of confirmed cases of introgression (Stewart *et al.* 2003).

Wild diploid and tetraploid members of *Zea* collectively referred to as teosinte are normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua; however, a fairly rare, sparsely dispersed feral population of teosinte has been reported in Florida. The Mexican and Central America teosinte populations primarily exist within and around cultivated maize fields; they are partially dependent on agricultural niches or open habitats, and in some cases are grazed upon or fed to cattle which distribute the seed. While some teosinte may be considered to be weeds in certain instances, they are also used by some farmers for breeding improved maize (Sánchez and Ruiz, 1997, and references therein). Teosinte is described to be susceptible to many of the same pests and diseases which attack cultivated corn (Sánchez and Ruiz, 1997).

All teosinte members can be crossed with cultivated corn to produce fertile F_1 hybrids (Doebley, 1990a; Wilkes, 1967). In areas of Mexico and Guatemala where teosinte and corn coexist, they have been reported to produce hybrids. Of the annual teosintes, *Z. mays* subsp. *mexicana* forms frequent hybrids with maize, *Z. luxurians* hybridizes only rarely with maize, whereas populations of *Z. mays* subsp. *parviglumis* are variable in this regard (Wilkes, 1977; Doebley, 1990a). Research on sympatric populations of maize and teosinte suggests introgression has occurred in the past, in particular from maize to *Z. mays* subsp. *luxurians* and *Z. mays* subsp. *diploperennis* and from annual Mexican plateau teosinte (*Z. mays* subsp. *mexicana*) to maize (Kato Y., 1997 and references therein).

Nonetheless, in the wild, introgressive hybridization from maize to teosinte is currently limited, in part, by several factors including distribution, differing degrees of genetic incompatibility, differences in flowering time in some cases, block inheritance, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Doebley, 1990a and 1990b; Galinat, 1988). First-generation hybrids are generally less fit for survival and dissemination in the wild, and show substantially reduced reproductive capacity which acts as a significant constraint on introgression. Teosinte has coexisted and co-evolved in close proximity to maize in the Americas over thousands of years, but maize and teosinte maintain distinct genetic constitutions despite sporadic introgression (Doebley, 1990a). The potential for gene introgression from MIR604 corn into teosinte would increase if varieties are developed, and approved for cultivation in locations where these teosintes are located. A limited potential can also occur through smuggling unapproved seeds or from import grain for planting. Since MIR604 corn does not exhibit characteristics that cause it to be any more weedy than other cultivated corn, its potential impact due to the limited potential for gene introgression into teosinte is not expected to be any different from that of other cultivated maize varieties.

The genus *Tripsacum* contains up to 16 recognized species, most of which are native to Mexico, Central and South America, but three of which exist as wild and/or cultivated species in the U.S. Though many of these species occur where corn might be cultivated, gene introgression from MIR604 corn under natural conditions is highly unlikely or impossible. Hybrids of *Tripsacum* species with *Zea* are difficult to obtain outside of a laboratory and are often sterile or have greatly reduced fertility, and none are able to withstand even the mildest winters. Furthermore, none of the sexually compatible relatives of corn in the U.S. are considered to be weeds in the

U.S. (Holm *et al.*, 1979), therefore, the unlikely acquisition of a single pesticide gene or the *pmi* gene would not be expected to transform them into weeds.

B. Potential impacts based on the relative weediness of MIR604 corn

APHIS assessed whether MIR604 corn is any more likely to become a weed than the nontransgenic recipient corn line, or other corn currently cultivated. The assessment encompasses a thorough consideration of the basic biology of corn and an evaluation of unique characteristics of MIR604 corn.

In the U.S., corn is not listed as a weed in the major weed references (Crockett, 1977; Holm *et al.*, 1979; Muenscher, 1980), nor is it present on the lists of noxious weed species distributed by the Federal Government (7 CFR Part 360). Furthermore, corn has been grown throughout the world without any report that it is a serious weed. Cultivated corn is unlikely to become a weed. It is not generally persistent in undisturbed environments without human intervention. Although corn volunteers are not uncommon, they are easily controlled by herbicides or mechanical means. Corn also possesses few of the characteristics of plants that are notably successful weeds (Baker, 1965; Keeler, 1989).

Syngenta conducted agronomic field trials at a total of 32 field trial locations in the U.S. Corn Belt during the 2002 and 2003 growing seasons. Table 4 (revised petition, page 58) identifies the traits assessed in the Agronomic Field Trials. For the majority of the traits assessed, there were no statistically significant differences between MIR604-derived hybrids and their negative segregant control counterparts. There were few statistically significant differences between the MIR604-derived hybrids and their negative segregant controls, as identified in Appendix 1C, Tables 1C to 4C and Appendix 1D, Tables 2D to 4D of the revised petition. Most of these differences were not consistent at the different sites over the two years of field trials. For example, one or both of the MIR604-hybrids exhibited a 'grain moisture percentage measured at harvest time' (GMSTP) that was significantly lower in the MIR604-derived hybrids at 9 field trial locations, significantly higher at 1 field trial location, and exhibited no difference at 10 of the field trials locations. At these 32 locations, the range of values for agronomic parameters, even when significantly different, was within the range of values expected for traditional maize hybrids. The results of these field trials indicate that MIR604 corn does not exhibit characteristics that would cause it to be more weedy than the parental corn line.

In addition, Syngenta conducted disease trials in 2002 and 2003, whereby MIR604 hybrids and their negative segregant controls were exposed to various corn pathogens, including Northern corn leaf blight (*Helminthosporium turcicum*), Southern corn leaf blight (*Helminthosporium maydis*), Eyespot (*Kabatiella zeae*), and Gray leaf spot (*Cercospora zeae-maydis*). Lesion density and spread were measured. No significant differences in disease susceptibility were found between line MIR604 corn and the non-transgenic counterparts (revised petition, Appendix 1C, Table 6C). The results of these trials indicate that MIR604 corn does not exhibit characteristics that would cause it to be more susceptible to disease than the parental corn line.

The introduced traits, coleopteran insect resistance and mannose utilization, are not expected to cause MIR604 corn to become a weed. Other CRW-resistant corn varieties previously

deregulated by APHIS did not exhibit characteristics that would enhance weediness (APHIS assessments are available at: http://www.aphis.usda.gov/brs/not_reg.html). None of the characteristics of weeds described by Baker (1965) involve resistance or susceptibility to insects, and there is no reason to expect that the protection against the target insects provided by this new corn line would release it from any constraint that would result in increased weediness. MIR604 corn is still susceptible to other insect pests and diseases of corn and it is unchanged in its susceptibility to injury by commercially available herbicides.

C. Potential impact on non-target organisms, including beneficial organisms and threatened or endangered species

APHIS evaluated the potential for line MIR604 corn plants and their products to have damaging or toxic effects directly or indirectly on non-target organisms. Non-target organisms considered were those representative of the exposed agricultural environment, including those that are recognized as beneficial to agriculture or as threatened or endangered in the U.S. APHIS also considered potential impacts on other "non-target" pests, since such impacts could potentially change agricultural practices.

The *pmi* (*manA*) gene comes from *E. coli* and encodes the enzyme phosphomannose isomerase (PMI). *Pmi* serves as a marker gene that enables selection of Bt lines, providing the plant with the ability to utilize mannose as a sole carbon source. Reports in the scientific literature support the conclusion that the expression of PMI protein in corn plants is not expected to have deleterious effects or significant impacts on non-target organisms, including beneficial organisms (Privalle, 2002). Additionally, the EPA has granted an exemption from the requirement of a tolerance for the PMI protein as an inert ingredient in all plants (U.S. EPA 2004a). The DNA encoding the PMI protein is not toxic. At the 80-amino acid peptide level, the PMI protein shares no significant homology with proteins known to be toxic or allergenic. Within one of the 80-amino acid windows, there was one region of sequence homology of eight contiguous amino acids between MIR604 PMI and a recently described allergen, α-parvalbumin, from *Rana* species (frog). Further testing found no cross-reactivity between the human serum Immunoglobulin E (IgE) and Bovine Serum Albumin (BSA), indicating that the low degree of sequence identity between MIR604 PMI and α-parvalbumin from *Rana* species is not biologically relevant.

Like the Cry1 class of insecticidal proteins, the specificity of the mCry3A protein insecticidal activity is dependent upon their binding to specific receptors present in the insect mid-gut (Lambert, *et al.*, 1996; Van Rie *et al.*, 1990; Van Rie *et al.*, 1989; Hofmann *et al.*, 1988a and 1988b; and Wolfersberger *et al.*, 1986). These insecticidal proteins are not expected to adversely affect other invertebrates or vertebrate organisms, including non-target birds, mammals and humans. APHIS evaluated laboratory and field studies on representative species that support these expectations. The toxicity and specificity of the coleopteran specific Cry proteins are associated with their solubilization and proteolytic activation in the insect midgut, and their binding to specific cell membrane receptors in the brush border membrane vesicles present in the midgut of susceptible insects. These specific receptors are not present in non-target species, including birds, mammals, and humans (Griffitts *et al.*, 2005; Lambert *et al.*, 1996; Van Rie *et al.*, 1990; Van Rie *et al.*, 1989; Hofmann *et al.*, 1988a and 1988b; and Wolfersberger *et al.*, 1986).

1. Potential impacts on target and non-target pests:

The mCry3A protein only has enhanced activity over the native Cry3A protein against select beetle (Order: Coleoptera) species within the family Chrysomelidae, namely corn rootworm. Syngenta conducted a series of diet bioassays with microbially-expressed mCry3A proteins to characterize the insecticidal specificity (see revised petition Chapter 7, Table 14, page 75). Test species included the target Coleopteran species: Northern corn rootworm (*D. barberi*), Western corn rootworm (*D. virgifera virgifera*), Southern corn rootworm (aka spotted cucumber beetle, *D. undecimpunctata howardi*); non-target Coleopteran pests: Colorado potato beetle (CPB; *Leptinotarsa decemlineata*), banded cucumber beetle (*Diaborotica balteata*) and cotton boll weevil (*Anthonoms grandis*). Additionally, test species also included lepidopteran pests, including: black cutworm (*Agrotis ipsilon*), corn earworm (*Heliocoverpa zea*), European corn borer (*Ostrinia nubilalis*), fall armyworm (*Spodoptera frugiperda*), pink bollworm (*Pectinophora gossypiella*) and tobacco budworm (*Heliothis virescens*). The microbially-expressed mCry3A protein exhibited activity against the following Coleopterans: CPB, Western corn rootworm, Northern corn rootworm, and banded cucumber beetle. For all but CPB, against which both the native Cry3A and mCry3A proteins were active, the mCry3A exhibited enhanced activity over the native Cry3A. However, neither the native nor mCry3A was active against the lepidopteran pests tested. Field trials of MIR604 corn plants also verified that corn plants expressing the mCry3A were better protected against NCRW and WCRW than nontransgenic corn plants (see revised petition Appendix 1A, Tables 2A and 3A) and MCRW (revised petition Appendix 1A, Table 5A). An additional glasshouse trial verified that MIR604-derived hybrids were better protected against NCRW than nontransgenic control hybrids (revised petition Appendix 1A, Table 4A). Unlike chemical insecticides, target species of corn rootworm species are only affected when they feed on the plants that express the mCry3A protein. Therefore, the use of MIR604 is unlikely to result in the extinction of these corn rootworm species.

2. Potential impacts on non-target organisms, including beneficial organisms:

The mCry3A protein is not expected to adversely affect non-target invertebrate and vertebrate organisms, including birds, mammals and humans, because they are not expected to contain the receptor found in the midgut of target insects. To evaluate the potential of line MIR604 corn to have damaging or toxic effects on representative terrestrial and an aquatic species, APHIS assessed data from a series of ecological toxicology experiments including the results of several studies submitted that were designed to evaluate the sensitivity of representative non-target organisms to mCry3A protein. Test substrates included corn plant material (e.g., corn grain, leaf or pollen) expressing mCry3A protein or protein purified from *E. coli* strain DH5α engineered to express the mCry3A protein. The mCry3A protein as extracted from the engineered DH5α was similar in its biochemical properties (molecular weight, amino acid sequence and lack of glycosylation) and in biological activity against WCRW to the mCry3A as produced in line MIR604 corn.

Acute dietary toxicity studies of beneficial arthropods were conducted in laboratory tests, and no adverse effects were observed at levels 10.6 to 36 times the estimated environmental exposure (EEC) calculated using estimates of corn consumption for each organism (revised petition, Table 19, page 87). MIR604 pollen does not contain detectable levels of mCry3A protein and therefore pollinators, like honey bees (*Apis mellifera*), will be exposed to negligible amounts of mCry3A.

However, standard test methods exist for larval *A. mellifera*, whereby deleterious effects of the test substance may be evaluated at a sensitive developmental stage. Beneficial natural enemies, insidious flower bug (aka minute pirate bug; *Orius insidiosus*) and the seven spotted lady beetle (*Coccinella septempunctata*) were fed microbially-produced protein mixed with artificial diet as well as representative ground-dwelling predators, a rove beetle species (*Aleochara bilineata*) and a ground beetle species (*Poecilus cupreus*). Since parasitic and predatory insects will have limited direct exposure to the mCry3A insecticidal protein expressed in line MIR604 corn, little impact is expected for these species other than a possible shift to alternate hosts since corn rootworm populations are expected to be reduced.

The sensitivity of other organisms to mCry3A was tested using microbially-expressed mCry3A protein. The additional organisms tested included earthworms (*Eisenia foetida*) as a representative decomposer, rainbow trout (*Oncorhynchus mykiss*), and bobwhite quail (*Colinus virginianus*). All of the organisms evaluated in the dietary toxicity studies were exposed to much greater levels of the mCry3A proteins than they would be exposed in the field (see revised petition, Chapter 7. Environmental Safety of mCry3A; Table 19, page 87) with no adverse effects observed.

In Chapter 9, Environmental Consequences of Introduction, the petitioner estimates that with the availability of MIR604 corn on the market, there could be a substantial reduction in the use of conventional pesticides, citing the potential elimination of 4.5 million acre pesticide treatments and approximately 1.25 million pounds of active ingredients within the first five years in sales of MIR604 corn. Tables 22 and 23 identify the corn rootworm pesticides by class currently used, including amount used (estimated pounds and percentage of use), acreage treated and grower cost. In general, mCry3A protein expressed in corn line MIR604 compares favorably to these products with respect to the reduced potential for harm in the environment.

3. Potential impacts on threatened and endangered arthropods:

APHIS coordinates review of petitions with other agencies that have regulatory oversight on these same products. With respect to threatened and endangered species, EPA also plays a role in the evaluation. Given the specificity of the mCry3A activity, species outside the insect order Coleoptera and family Chrysomelidae should not be affected. There are no endangered Chrysomelidae within the U.S. APHIS has thoroughly examined all threatened and endangered coleopterans that occur in counties where corn is grown, and determined that the breeding habitat of coleopterans does not put them in proximity of corn fields.

In addition to the expected lack of toxicity to coleoptera other than those in the family Chrysomelidae, it is very unlikely that endangered species of coleopteran will be exposed to the mCry3A protein expressed in MIR604. Many of the endangered and threatened beetles occur in cave or aquatic habitats. None of the endangered beetles are expected to occur in or near cornfields. The American burying beetle (*Nicrophorus americanus*) may occur in old fields or cropland hedge rows. However, based upon the feeding habits of the American burying beetle, it is not expected to occur within cornfields nor will it be exposed to mCry3A protein. Adult American burying beetles are classified as opportunistic scavengers that feed on anything dead and bury vertebrate carcasses which larvae feed on. Larvae is fed carrion that is regurgitated by adults until the larvae are able to feed directly on a carcass. Hungerford's crawling water beetle (*Brychius hungerfordi*) may be found in cool riffles of clean, slightly alkaline streams of Michigan and Ontario, Canada. APHIS has concluded that this beetle is unlikely to be exposed to mCry3A, because it typically prefers well aerated streams of moderate to fast flow, where it feeds primarily on algae growing on rocks.

There is indirect evidence that supports Syngenta's assertion of the specificity of the mCry3A insecticidal properties and further suggests that mCry3A proteins would not impact Hungerford's crawling water beetle. Results of feeding studies included within the petition indicate that mCry3A is toxic only within the specific coleopteran suborder of Polyphaga that includes the target CRW species. These data indicate that other coleopterans experience no significant level of toxicity from mCry3A. Thus, toxicity testing reveals that *Poecilus cupreus*, which is in the same suborder as Hungerford's crawling water beetle, specifically Adephaga, is not sensitive to mCry3A, and additionally, its habitat preferences detailed above make exposure to mCry3A extremely unlikely. In the event that MIR604 plant tissue or mCry3A proteins from cornfield soils entered into these riparian environments, it would be quickly dissipated and exposure would be negligible. While the American burying beetle is in the same suborder as CRW, its exposure to the mCry3A proteins would be extremely unlikely due to the habitat preference described above.

BRS has reviewed the data in accordance with a process mutually agreed upon with the U.S. Fish and Wildlife Service (FWS) to determine when a consultation, as required under section 7 of the Endangered Species Act, is needed. APHIS has reached a determination that the release following a determination of nonregulated status would have no effects on listed threatened or endangered species or their critical habitat and consequently, consultation with FWS is not required for this EA.

4. Environmental fate in soil:

The purpose of the soil fate study was to test the inherent degradability of mCry3A in a soil typical of corn-growing areas and with healthy microbial activity.

An insect bioassay was conducted in the laboratory with the Colorado potato beetle (CPB) to determine the DT_{50} (time to 50% degradation) of the mCry3A protein in soil. APHIS determined that Syngenta's soil study and use of the bacterially-produced mCry3A, which Syngenta demonstrated in its petition to be biochemically and biologically similar to the protein in corn tissues, was sufficient to evaluate the environmental fate of mCry3A in soil. Mortality of the CPB was monitored and modeled using first-order kinetics to determine the DT_{50}. The laboratory bioassay established a DT_{50} of 7.6 days. This is approximately 50% shorter than that of one of the commonly used insecticides currently used to control corn rootworms (Appendix 2).

The study, which was conducted under Good Laboratory Practices, estimated the soil DT_{50} of mCry3A from Colorado potato beetle bioassays using standard analytical methods for soil fate studies of *Bt* proteins. Most proteins do not persist or accumulate in soil because they are degraded by soil proteases. Degradation of mCry3A in a live soil showed that it is not resistant to degradation by soil proteases and therefore is unlikely to persist or accumulate in the field. Field studies with transgenic cotton and corn have shown that laboratory degradation studies are good predictors of the behavior of Cry proteins in the field. The study therefore met the Agency's standards and the EPA accepted the results of the study (U.S. EPA, 2007). APHIS notes that while it has determined that there is sufficient evidence to reasonably conclude that there will not be a significant environmental impact on the soil environment by granting nonregulated status in whole to MIR604 due to limited persistence of mCry3A, EPA has requested that the applicant submit additional studies to evaluate insecticidal protein degradation, accumulation, and persistence in a variety of soil types, with sampling conducted each year for three years (U.S. EPA BRAD, 2007).

D. Potential impacts on biodiversity

Our analysis concludes that line MIR604 corn exhibits no traits that would cause increased weediness, that its unconfined cultivation should not lead to increased weediness of other cultivated corn or other sexually compatible relatives, and that it is unlikely to harm non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service.

The importance of corn as a food crop, and its dependence on human management, has produced a long history of great care to protect germplasm lines of corn. Decades prior to the introduction of transgenic corn products, the corn industry developed effective methods and means to maintain product segmentation and genetic purity standards. Specialty corns, for example, were successfully isolated for years and continue to be grown today, even with transgenic corn widely adopted in the U.S. Moreover, with respect to both conventional and transgenic corn, the ability to protect and maintain the genetic purity of breeding lines is critical to seed companies and developers of new varieties such as MIR604. Consequently, seed companies routinely apply standard breeding techniques – including physical and temporal isolation – that have proven effective at maintaining the genetic purity of breeding lines. Genetically engineered lines with Bt traits have been available on the market since 2002 and have not had a negative effect on biodiversity. The granting of nonregulated status in whole to MIR604 is not expected to cause additional and significant environmental impacts to biodiversity.

Based on this analysis, there is no apparent potential for significant impact to biodiversity. If

APHIS chooses the no action alternative, there would also be no impact on biodiversity.

E. Potential impacts on agricultural and cultivation practices

APHIS considered potential impacts associated with the cultivation of rootworm-resistant corn line MIR604 on current agricultural practices, in particular, those used to control CRW in corn. The potential impact on organic farming was also considered.

1. Potential impacts of line MIR604 corn on insect control practices

Syngenta has provided data which indicate that MIR604 corn expresses the mCry3A protein in root tissues to provide control of corn rootworms. The availability of this product is likely to have an impact on current control practices for corn rootworm that include the use of crop rotation, chemical insecticides, and other Bt corn varieties that are intended to control corn rootworm. Both crop rotation and the use of chemical insecticides have been important strategies in the past. However, CRW have developed several adaptations to control methods including crop rotation and insecticide resistance. Since CRW predominantly oviposit in cornfields, rotating corn with small grains, hay, clover or alfalfa has been utilized as a control method (Levine and Oloumi-Sadeghi 1991).

Soybean rotation was formerly an effective strategy to control CRW in corn and thereby minimize pesticide application. However, WCRW has developed an adaptation to resist the corn/soybean rotation in Illinois and Indiana (Levine and Oloumi-Sadeghi 1996). In areas such as east-central Illinois and northern Indiana, the WCRW has been found to have the ability to lay eggs in soybean, overwinter and hatch the following year in corn (Levine and Oloumi-Sadeghi 1991, Levine and Oloumi-Sadeghi 1996, O'Neal *et al.* 1999, Isard *et al.* 1999, Isard *et al.* 2000). Northern CRW populations have also developed resistance to the corn/soybean rotation in Minnesota, Iowa, and South Dakota (Gray *et al.* 1998). Prolonged diapause of NCRW involves eggs that remain viable for two winters and hatch two seasons after being laid. Northern CRW have developed the ability for prolonged or extended diapause resulting in a significant proportion of their eggs hatching after two winters leading to an adaptation to rotating corn with crops such as soybean. Extended diapause has been verified in the laboratory from NCRW eggs collected from South Dakota, Minnesota, Illinois and Michigan (Krysan *et al.* 1984, Krysan *et al* 1986, Levine and Oloumi-Sadeghi 1991 Levine *et al.* 1992a, Levine *et al.* 1992b). Field studies conducted by Tollefson (1988) in northwestern Iowa cornfields suggest that extended diapause occurs throughout NCRW distribution in rotated fields. Another study conducted by Levine and Oloumi-Sadeghi (1996) suggests that the WCRW does not demonstrate extended diapause. In these cases, resistance took at least ten and usually more than 15 years to develop without implementing insect resistance management (IRM) strategies. Instances of CRW resistance to crop rotation and/or insecticide use typically develop on a local scale which is probably due to limited adult movement before and after mating. Research is currently underway at the University of Nebraska and USDA-ARS in North Dakota to determine the genetics of esterase-mediated insecticide resistance in WCRW populations. Results of this research are intended to provide knowledge on localized selection and migration that may aid in refining future IRM strategies.

In addition to the problem with insect adaptation to crop rotation, many growers simply prefer to grow corn continuously, a practice which necessitates higher inputs of chemical insecticides. In

2001, about 18% of all corn acres were treated for CRW with insecticides. However, producers growing continuous corn had a much higher incidence of soil insecticide use; with about 38% of these acres treated with insecticides for CRW (Payne et al. 2003).

With crop rotation losing its effectiveness to provide adequate CRW control, the primary alternative to insect-resistant GE corn is traditional insecticide use. More than nine million pounds of insecticide were applied to the 2001 US corn crop (Payne et al. 2003). The most widely used insecticides are from the organophosphate or synthetic pyrethroid classes of chemistry. It is therefore expected that availability of another practical and economical alternative to chemical insecticides for CRW control would result in a significant reduction in application of such chemicals. Syngenta has provided data which indicate that MON 863 corn expresses modified Cry3Bb protein in root tissues to provide control of corn rootworms. The availability of this product is likely to have an impact on current control practices for corn rootworm. Both crop rotation and the use of chemical insecticides are important strategies. Crop rotation has been effective while being environmentally favorable. But chemical control remains an important strategy also for several reasons. One reason is extended diapause in which some eggs can survive through the non-corn rotation to attack corn in a subsequent season (Ostlie, 1987; Tollefson, 1988; Gray et al., 1998). Extended diapause has been observed for both the NCRW and the WCRW . Another is that a new strain has developed in central Illinois and northern Indiana that can survive and replicate on soybean, the crop most often rotated with corn in the corn belt (Onstad and Joselyn, 1999; O=Neal et al., 1999). This strain has spread rapidly since it was first observed in 1993 and it is expected to continue to spread throughout the corn belt. And yet another reason is that many growers simply prefer to grow corn continuously, a practice which necessitates higher inputs of chemical insecticides. As a result of these factors and the very damaging nature of the pest, chemical insecticide usage has increased. The most common chemical regime is the application of a granular insecticide at planting, either banded or in-furrow. In some cases sprays are applied for adult suppression. The USDA National Agricultural extension Service (NASS) statistics compiled from 15 top corn producing states in the Midwest indicate that 30% of this acreage were treated with insecticide registered for corn rootworm control. It is difficult to surmise how much of this application was for the corn rootworm control as these insecticides these products used alone or in combination also control other pests such as black cutworms. A 1995 survey conducted in Iowa, the leading corn producing state which accounts for 17.5% of all U.S. production, indicated that growers used chemicals to control CRW 22 % of the time. The most widely used insecticides are from the organophosphate or synthetic pyrethroid classes of chemistry. It is therefore expected that availability of a practical and economical alternative to chemical insecticides for CRW control would result in a significant reduction in application of such chemicals.

The EPA has produced a number of documents regarding the use of Bt technology in corn. A risks and benefits assessment for reregistration of Bt corn and cotton plant incorporated protectants (PIP's) has been prepared by the EPA (U.S. EPA, 2000) and is posted at the following EPA internet site: http://www.epa.gov/scipoly/sap/meetings/2000/index.htm. Issues considered by the EPA pertaining to this assessment were the subject of a meeting convened on October 18-20, 2000, by the EPA Federal Scientific Advisory Panel (SAP). In 2001, EPA issued a registration document for *Bacillus thurigiensis* Plant-incorporated protectants. In this document, EPA confirms their original findings that "there are no unreasonable adverse health

effects from these products" and that there are no unreasonable adverse effects in corn on nontarget wildlife or beneficial organisms (US EPA, 2001). EPA also convened a SAP meeting, August 27-29, 2002, to consider issues related to corn rootworm-related PIP's. The results of this SAP meeting can be found at: http://www.epa.gov/scipoly/sap/2002/index.htm. An SAP was also held for MIR604 corn on March 14-15, 2006 (http://www.epa.gov/scipoly/sap/meetings/2006/march/finalmeetingminutes6_1_2006.pdf).

Before these new Bt corn varieties were available, farmers were willing to accept lower corn yields, rather than incur the expense, trouble, and uncertain results of chemical insecticide applications to control the target pests. With Bt seed technology, each individual plant is protected, resulting in reduced insecticide use where insecticides are used to control for CRW, lower labor costs and increased yields during significant CRW infestation relative to non-Bt fields (Payne et al. 2003). Following the registration of Bt corn varieties in 1995, growers were quick to embrace the new technology. Estimates of Bt corn acreage as a percent of total corn acreage planted increased from 1% in 1996 to 40%* in 2006 (USDA NASS summarized at http://www.ers.usda.gov/data/biotechcrops/ExtentofAdoptionTable1.htm).

MIR604 corn could be incorporated into current integrated pest management (IPM) practices as an additional tool for control. Fields are typically scouted for adult CRW in the late summer or early fall. Economic thresholds are then used in making decisions about control strategies for the following spring planting season. MIR604 offers an alternative to organophosphate and pyrethroid insecticide applications in cases where thresholds indicate CRW control is needed and the grower chooses to grow corn. No new or specialized equipment or skills would be needed to use the new technology. Reduced pesticide usage by the growers would carry the accompanied benefits of reduced needs for the manufacture, transport, storage and disposal of hazardous chemicals and containers.

In order to delay the potential evolution of resistance in the target pests to Bt Cry proteins expressed in plants, growers have been required by the EPA and/or the developers to implement insect resistance management (IRM) strategies. Syngenta has submitted to EPA a detailed strategy for approval prior to commercialization of this product. The plan includes monitoring for compliance with the IRM plant, grower education, monitoring for resistance to development of resistant CRW populations and mitigation measures if resistant populations are confirmed. Such insect management strategies may be responsible, in part, for delaying the development of resistance to the Cry toxins. Cry3Bb1 corn has been registered by EPA for commercial production since 2002 and there have been no reports of coleopteran insect resistance developing in the field to any Bt toxin expressed in any plant. Considering the implementation of these insect resistance management strategies and their apparent effectiveness in delaying or preventing the development of resistance in CRW populations to the Cry protein, APHIS feels that the granting of non-regulated status to MIR604 corn will not significantly impact the environment by causing the development of resistance in this target pest species.

2. Potential impacts of line MIR604 corn on weed control

APHIS evaluated data submitted by the petitioner that show that hybrids derived from line

* 40% value is the sum of insect resistant (Bt) corn and stacked varieties (varieties with herbicide tolerant and insect resistant traits)

MIR604 corn express mCry3A. Line MIR604 corn is expected to have no impact on current agricultural practices used for weed control as it is no more herbicide tolerant than its nonengineered counterpart.

Volunteers of line MIR604 corn can be controlled by selective mechanical or manual weed removal or by the use of several commercially available herbicides. For example, in soybean, which is the crop most commonly rotated with corn, herbicides based on sulfonylurea, lipid biosynthesis inhibitors, or Fluazifop/fomesafen could be used to control maize volunteers. The commercial introduction and wide adoption in the United States of Roundup Ready® soybeans has been associated with an increase in the use of glyphosate to control weeds in soybean, while the use of other herbicides has decreased (Fernandez-Cornejo and McBride, 2000; Heimlich *et al.*, 2000). Glyphosate could also be used to control volunteers of line MIR604 corn in Roundup Ready® soybeans. It is estimated that in 1996, 7% of the total soybean acreage was planted to herbicide tolerant soybeans, compared to an estimated 82% of total soybean acreage planted to herbicide tolerant soybeans in 2003 (Sankula and Blumenthal, 2004). Additionally, glufosinate could be used. Both glyphosate and glufosinate have relatively low toxicity to humans and wildlife, and do not persist in the environment (Pike, 1999; McGlamery *et al.* 1999).

3. Potential impacts on organic farming

The National Organic Program (NOP) is administered by USDA's Agricultural Marketing Service (AMS). Organic production operations must develop and maintain an organic production system plan approved by their accredited certifying agent in order to obtain certification. Organic certification of a production or handling operation is a process claim, not a product claim. Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. Oversight by a certifying agent includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records.

The organic system plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. Excluded methods include a variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods, unless a certifying agent has reasonable suspicion that a prohibited substance or excluded method was used. The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards.

It is not likely that organic farmers, or other farmers who choose not to plant transgenic varieties or sell transgenic grain, will be significantly impacted by the expected commercial use of this product since: (a) nontransgenic corn will likely still be sold and will be readily available to those who wish to plant it; (b) farmers purchasing seed will know this product is transgenic because it will be marketed as *Bt mCry3A* coleopteran resistant; and (c) based on the IRM plan, farmers will be educated about recommended management practices. Transgenic corn lines resistant to coleopteran insects, and/or tolerant to glufosinate are already in widespread use by farmers. This particular product should not present new and different issues than those with

respect to impacts on organic farmers. APHIS has considered that corn is open-pollinating and it is possible that the engineered genes could move via wind-blown pollen to an adjacent field. All corn, whether genetically engineered or not, can transmit pollen to nearby fields, and a very small influx of pollen originating from a given corn variety does not appreciably change the characteristics of corn in adjacent fields. As described previously in this assessment, the rate of cross-pollination from one field to another is expected to be quite low, even if flowering times coincide. The frequency of such an occurrence decreases with increasing distance from the pollen source such that it sufficiently low at 660 feet away to be considered adequate for production of certified corn seeds. Methods of spatial and temporal isolation are widely used when seed producers are seeking to minimize the influx of pollen from sources outside the seed production field. These methods are readily applicable for the production of certified organic corn seed.

F. Potential impacts on raw or processed agricultural commodities.

APHIS analysis of data on agronomic performance, disease and insect susceptibility, and compositional profiles of the kernels indicate no differences between MIR 604 and their non-transgenic hybrid counterparts that would be expected to cause either a direct or indirect plant pest effect on any raw or processed plant commodity from deregulation of line MIR604.

G. Cumulative Impacts

APHIS considered whether the proposed action could lead to significant cumulative impacts, when considered in light of other past, present, and reasonably foreseeable future actions, regardless of what agency or person undertakes such actions. MIR604 is not the first Bt corn product to be granted nonregulated status. APHIS has previously made determinations of nonregulated status as to other Bt corn products. *See* Determination of Nonregulated Status for MON863, 67 Fed. Reg. 65,087 (Oct. 23, 2002); Approval of Mycogen Seeds/Dow AgroSciences LLC and Pioneer Hi-Bred Int'l, Inc. Request No. 03-353-01p Seeking a Determination of Non-regulated Status for *Bt* Cry34Ab1/35Ab1 Insect Resistant, Glufosinate Tolerant Corn Line 59122-7 (Sept. 23, 2005)[†]; Approval of Monsanto Company Request 04-125-01p Seeking a Determination of Non-regulated Status for Corn Rootworm Resistant Corn MON 88017 (Dec. 14, 2005).[‡] APHIS evaluated the potential cumulative impacts of granting nonregulated status, in whole, to MIR604 corn. It considered how MIR604 would alter corn cultivation, genetic diversity of corn as well as the development insect resistance.

1. Specialization of Corn Cultivation Has Been Maintained Through Multiple Bt Corn Events.

Maintaining genetic purity has been a feature of corn cultivation for decades as part of hybrid seed and specialty corn production, and multiple Bt corn events have not significantly affected these processes, even considering the effects of these transgenic events cumulatively. Since the adoption of hybrid corn in the 1930s, corn production has required separation of inbred parent and hybrid seed production activities from the production of grain. This is required to maintain genetic purity of inbred parents and guarantee the quality of hybrid seed sold to corn growers.

[†] *Available at* http://www.aphis.usda.gov/brs/aphisdocs2/03_35301p_com.pdf.

[‡] *Available at* http://www.aphis.usda.gov/brs/aphisdocs2/04_12501p_com.pdf.

Many methods are used effectively for this purpose, including the following: maintaining isolation distances to prevent pollen movement from other corn, planting border or barrier rows to intercept pollen, employing natural barriers to pollen movement such as treelines, manual or mechanical detasseling, genetic male sterility, and staggered planting dates. Similar to the production of conventional inbred and hybrid seed, industry quality standards for specialty corn products have led specialty corn seed producers and growers to employ a variety of techniques to ensure that their products are not pollinated by or commingled with conventional field corn. In general, all the management practices used in conventional seed production to ensure quality standards are also employed in, and are sufficient to meet standards for, the production of specialty corn seed.

Prior to the introduction of transgenic corn products, the corn industry developed effective methods and means to maintain product segmentation and genetic purity standards. As a result, these widespread practices have served to ensure that the broad adoption of transgenic corn in the U.S. (including the sale and cultivation of multiple Bt corn varieties over more than a decade) has had no significant impact, even in the aggregate, on the production of corn seed and specialty corn products. APHIS does not foresee a cumulative impact from granting nonregulated status in whole to MIR604 corn.

2. Genetic Diversity of Corn Has Been Preserved Following Multiple Bt Corn Events.

The adoption of multiple varieties of transgenic corn has had no significant impact on the genetic diversity of cultivated corn or the availability of diverse corn germplasm resources, even considering the effects of these transgenic events cumulatively. Genetically distinct corn hybrids have always been developed for various geographies and purposes, and are continually improved by plant breeding. This has in no way been altered by transgenic corn—transgene events are simply incorporated into these breeding programs, and have not obviated the continuous improvement of the base genetics that underlie the performance of modern corn hybrids.

In addition, the adoption of transgenics was preceded by worldwide efforts to identify and preserve sources of maize genetic diversity, and to make these resources available for utilization by public and private corn breeders. Among these efforts are the Germplasm Enhancement of Maize program ("GEM"), a cooperative effort undertaken by USDA, public and private plant sector breeders, NGOs and international public cooperators, which was established to further identify corn genetic diversity and to provide it in useful form in order to broaden the genetic base of this crop.[§] The germplasm sources being developed through GEM are available free of charge through the extensive national germplasm collections and germplasm repository programs for conservation of corn genetic diversity.

Thus, observation of numerous other transgenic corn products indicates that the genetic diversity of corn has been maintained in coexistence with these events. APHIS does not foresee a significant cumulative impact on the genetic diversity of corn.

[§] USDA 1999. Germplasm enhancement of maize. Agricultural Research Service, Washington D.C. (http://www.public.iastate.edu/~usda-gem/corn.html).

3. Multiple Bt Corn Events Have Resulted in No Documented Insect Resistance Developing in the Field.

There have been no documented instances of confirmed insect resistance in natural populations of target insects to Bt corn or the Cry toxins they produce, despite the introduction of multiple previous events over the past decade. All commercialized Bt corn products are subject to mandatory refuge requirements as part of the terms of registration as plant-incorporated protectants by EPA. The fact that there have been no documented instances of confirmed insect resistance to Bt corn in the field indicates that the use of mandatory refuges is effective in preventing or delaying the development of insect resistance to Bt, even cumulatively after multiple Bt corn event introductions.

APHIS does not foresee a cumulative impact that would result in the development of insect resistance as EPA requires IRM for all registered crops expressing pesticidal compounds.

VII. Consideration of Executive Orders, Standards and Treaties Relating to Environmental Impacts

Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.

EO 13045, “Protection of Children from Environmental Health Risks and Safety Risks,” acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency’s mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children.

Each alternative was analyzed with respect to EO 12898 and 13045. None of the alternatives are expected to have a disproportionate adverse effect on minorities, low-income populations, or children. Collectively, the available mammalian toxicity, along with the history of safe use of microbial Bt products and other corn varieties expressing Bt proteins, establishes the safety of corn line MIR604 and its products to humans, including minorities, low income populations, and children who might be exposed to them through agricultural production and/or processing. No additional safety precautions would need to be taken. None of the impacts on agricultural practices expected to be associated with deregulation of corn line MIR604 described above are expected to have a disproportionate adverse effect on minorities, low income populations, or children. As noted above, the cultivation of previously deregulated corn varieties with similar insect resistance traits has been associated with a decrease and/or shift in pesticide applications for those who adopt these varieties that is either favorable or neutral with respect to environmental and human toxicity. If pesticide applications are reduced, there may be a beneficial effect on children and low income populations that might be exposed to the chemicals.

These populations might include migrant farm workers and their families, and other rural-dwelling individuals who are exposed to pesticides through ground-water contamination or other means of exposure. It is expected that EPA and USDA Economic Research Service would monitor the use of this product to determine impacts on agricultural practices such as chemical use as they have done previously for Bt products.

EO 13112, "Invasive Species", states that Federal agencies take action to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause. Nonengineered corn as well as other Bt and herbicide tolerant corn varieties are widely grown in the U.S. Based on historical experience with these varieties and the data submitted by the applicant and reviewed by APHIS, the engineered plant is sufficiently similar in fitness characteristics to other corn varieties currently grown, and it is not expected to have an increased invasive potential.

Executive Order 12114, "Environmental Effects Abroad of Major Federal Actions" requires Federal officials to take into consideration any potential environmental effects outside the U.S., its territories and possessions that result from actions being taken. APHIS has given this due consideration and does not expect a significant environmental impact outside the U.S. should nonregulated status be determined for corn line MIR604 or if the other alternatives are chosen. It should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new corn cultivars internationally, apply equally to those covered by an APHIS determination of nonregulated status under 7 CFR Part 340. Any international traffic in MIR604 corn subsequent to a determination of non-regulated status for line MIR604 would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC).

The purpose of the IPPC "is to secure a common and effective action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control" (http://www.ippc.int/IPP/En/default.htm). The protection it affords extends to natural flora and plant products and includes both direct and indirect damage by pests, including weeds. The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (116 countries as of June, 2001). In April, 2004, a standard for pest risk analysis of living modified organisms (LMOs) was adopted at a meeting of the governing body of the IPPC as a supplement to an existing standard, International Standard for Phytosanitary Measure No. 11 (ISPM-11; Pest Risk Analysis for Quarantine Pests). The standard acknowledges that all LMOs will not present a pest risk, and that a determination needs to be made early in the PRA for importation as to whether the LMO poses a potential pest risk resulting from the genetic modification. APHIS pest risk assessment procedures for bioengineered organisms are consistent with the guidance developed under the IPPC. In addition, issues that may relate to commercialization and transboundary movement of particular agricultural commodities produced through biotechnology are being addressed in other international forums and through national regulations.

The Cartagena Protocol on Biosafety is a treaty under the United Nations Convention on Biological Diversity (CBD) that established a framework for the safe transboundary movement,

with respect to the environment and biodiversity, of LMOs, which includes those modified through biotechnology. The Protocol came into force on September 11, 2003 and 119 countries are parties to it as of April 14, 2005 (see http://www.biodiv.org/biosafety/default.aspx). Although the U.S. is not a party to the CBD, and thus not a party to the Cartagena Protocol on Biosafety, U.S. exporters will still need to comply with domestic regulations that importing countries that are parties to the Protocol have put in place to comply with their obligations. The first intentional transboundary movement of LMOs intended for environmental release (field trials or commercial planting) will require consent from the importing country under an advanced informed agreement (AIA) provision, which includes a requirement for a risk assessment consistent with Annex III of the Protocol, and the required documentation. LMOs imported for food, feed or processing (FFP) are exempt from the AIA procedure, and are covered under Article 11 and Annex II of the Protocol. Under Article 11 Parties must post decisions to the Biosafety Clearinghouse database on domestic use of LMOs for FFP that may be subject to transboundary movement. To facilitate compliance with obligations to this protocol, the US Government has developed a website that provides the status of all regulatory reviews completed for different uses of bioengineered products (http://usbiotechreg.nbii.gov). These data will be available to the Biosafety Clearinghouse.

APHIS continues to work toward harmonization of biosafety and biotechnology consensus documents, guidelines and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the U.S. and in the Organization for Economic Cooperation and Development (OECD). NAPPO has completed three modules of a standard for the *Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries* (see http://www.nappo.org/Standards/Std-e.html). APHIS also participates in the North American Biotechnology Initiative (NABI), a forum for information exchange and cooperation on agricultural biotechnology issues for the U.S., Mexico and Canada. In addition, bilateral discussions on biotechnology regulatory issues are held regularly with other countries including: Argentina, Brazil, Japan, China, and Korea. Many countries, e.g. Argentina, Australia, Canada, China, Japan, Korea, Philippines, South Africa, Switzerland, the United Kingdom, and the European Union have already approved Bt corn varieties to be grown or imported for food or feed (http://www.agbios.com/dbase.php).

VIII. Literature Cited

Baker, H. G. 1965. Characteristics and modes of origin of weeds, pp. 147-168. *In:* The Genetics of Colonizing Species. (eds. Baker, H. G. and Stebbins, G. L.), Academic Press, New York.

Beadle, G. 1980. The ancestry of corn. Sci. Am. 242:112-119.

Crockett, L. 1977. Wildly Successful Plants: North American Weeds. University of Hawaii Press, Honolulu, Hawaii. 609 pp.

Doebley, J. 1990a. Molecular evidence for gene flow among *Zea* species. BioScience 40:443-448.

Doebley, J. 1990b. Molecular systematics of *Zea* (Gramineae). Maydica 35(2):143-50.

Dubelman, S. Ayden, B.R., Bader, B.M., Brown, C.R., Jiang, C., and Vlachos, D. (2005) Cry1Ab protein does not persist in soil after 3 years of sustained Bt corn use. Environmental Entomology 34: 915-921.

Fernandez-Cornejo, J. and McBride, W. D. [with contributions from Klotz-Ingram, D., Jans, S., and Brooks, N.] 2000. Genetically Engineered Crops for Pest Management in U.S. Agriculture: Farm-Level Effects. U.S. Department of Agriculture, Economic Research Service, Resource Economics Division. Agricultural Economic Report No. 786.

Galinat, W. C. 1988. The Origin of Corn, pp. 1-31. *In*: Corn and Corn Improvement, Third Edition. (eds. Sprague, G. F. and Dudley, J. W.). American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, Wisconsin.

Gianessi, L. P, Silvers, C. S., Sankula, S., and Carpenter, J. E. 2002. Plant Biotechnology: Current and Potential Impact for Improving Pest Management in U.S. Agriculture – An Analysis of 40 Case Studies: Insect Resistant Field Corn (3) – www.ncfap.org.

Gray, M. E, Levine, E., and Oloumi-Sadeghi, H. 1998. Adaptation to crop rotation: western and northern corn rootworms respond uniquely to cultural practice. Recent Develop. Entomol. 2:19-31.

Head, G., Surber, J., Watson, J., Martin, J., Duan, J. (2002) No detection of Cry1Ac protein in soil after multiple years of Bt cotton (Bollgard) use. *Environmental Entomology* 31: 30-36.

Heimlich, R. E., Fernandez-Cornejo, J., McBride, W., Klotz-Ingram, C., Jans, S., and Brooks, N. 2000. Genetically Engineered Crops: Has adoption reduced pesticide use? Agricultural Outlook, Economic Research Service/USDA. August 2000: 13-17.

Hitchcock, A. S. (revisions by Agnes Chase) 1971. *Tripsacum* L. Gamagrass, *In* Manual of the Grasses of the United States. Miscellaneous Publication 200, U.S. Department of Agriculture, 2nd ed., pp 790-792, Dover, NY.

Hofmann, C., Luthy, P., Hutter, R., and Piska, V. 1988a. Binding of the delta endotoxin from *Bacillus thuringiensis* to brush-border membrane vesicles of the Cabbage Butterfly (*Pieris brassicae*). Eur. J. Biochem. 173:85-91.

Hofmann, C., Vanderbruggen, H., Hofte, H., Van Rie, J., Jansens, S., and Van Mellaert, H. 1988b. Specificity of *B. thuringiensis* delta-endotoxins is correlated with the presence of high affinity binding sites in the brush-border membrane of target insect midguts. Proc. Natl. Acad. Sci. USA 85:7844-7448.

Holm, L., Pancho, J. V., Herbarger, J. P., and Plucknett, D. L. 1979. A Geographical Atlas of World Weeds. John Wiley and Sons, New York. 391 pp.

Isard, S. A., Spencer, J. L., Nasser M. A., and Levine, E. 2000. Aerial movement of western

corn rootworm (Coleoptera: Chrysomelidae): diel periodicity of flight activity in soybean fields. Environ. Entomol. 29(2): 226-234.

Isard, S. A., Nasser, M. A., Spencer, J. L. and Levine, E. 1999. The influence of weather on western corn rootworm flight activity at the borders of a soybean field in east central Illinois. Aerobiologia. 15: 95-104.

Kato Y., T. A. 1997. Review of introgression between maize and teosinte. *In:* Gene Flow Among Maize Landraces, Improved Maize Varieties, and Teosinte: Implications for Transgenic Maize. (eds. Serratos, J.A., Willcox, M.C., and Castillo-González, F.) pp. 44-53. Mexico, D.F., CIMMYT.

Keeler, K. 1989. Can genetically engineered crops become weeds? Bio/Technology 7:1134-1139.

Krysan, J. L. , Foster, D. E., Branson, T. F., Ostlie, K. R. and Cranshaw, W.S.. 1986. Two years before the hatch: rootworms adapt to crop rotation. Bull. Entomol. Soc. Amer. 32: 250-253.

Krysan, J. L., Jackson, J. J., and Lew, A. C. 1984. Field termination of egg diapause in *Diabrotica* with new evidence of extended diapause in *D. barberi* (Coleoptera: Chrysomelidae). Environ. Entomol. 13: 1237-1240.

Lambert, B., Buysse, L., Decock, C., Jansens, S., Piens, C., Saey, B., Seurinck, J., Van Audenhove, K., Van Rie, J., Van Vliet, A., and Peferoen, M. 1996. A *Bacillus thuringiensis* insecticidal crystal protein with a high activity against members of the family Noctuidae. Appl. Envir. Microbiol. 62(1):80-86.

Levine, E. and Oloumi-Sadeghi, H. 1991. Management of Diabroticite rootworms in corn. Annu. Rev. Entomol. 36:229-255.

Levine, E. and Oloumi-Sadeghi, H. 1996. Western corn rootworm (Coleoptera: Chrysomelidae) larval injury to corn for seed production following soybean grown for seed production. J. Econ. Entomol. 89: 1010-1016.

Levine, E., Oloumi-Sadeghi, H., and Fisher, J. R. 1992a. Discovery of multiyear diapause in Illinois and South Dakota northern corn rootworm (Coleoptera: Chrysomelidae) eggs and incidence of the prolonged diapause trait in Illinois. J. Econ. Entomol. 85(1): 262-267.

Levine, E., Oloumi-Sadeghi, H., and Ellis, C. R.. 1992b. Thermal requirements, hatching patterns, and prolonged diapause in western corn rootworm (Coleoptera: Chrysomelidae) eggs. J. Econ. Entomol. 85(6): 2425-2432.

Marx, M-C. Kandeler E., Wood M., Wermbter N. and Jarvis S.C. (2005) Exploring the enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. *Soil Biology and Biochemistry* 37: 35-48

McGlamery, M., Hager, A., and Sprague, C. 1999. Toxicity of Herbicides. Chapter 16, pp. 287-290. *In:* 2000 Illinois Agricultural Pest Management Handbook. College of Agricultural, Consumer and Environmental Sciences, University of Illinois at Urbana-Champaign. University of Illinois Board of Trustees. ISBN 1-883097-25-8. Available at: *http://www.ag.uiuc.edu/%7Evista/abstracts/aiapm2k.html*

Muenscher, W. C. 1980. Weeds. Second Edition. Cornell University Press, New York and London. 586 pp.

NASS. 2000. National Agricultural Statistics Service, United States Department of Agriculture. Agricultural chemical usage, 1999 field crops summary, report AG CH 1 (00)a; http://usda.mannlib.cornell.edu/usda/

Norman, M. J. T., Pearson, C. J., and Searle, P. G. E. 1995. The ecology of tropical food crops. Cambridge University Press, Cambridge, Great Britain. pp 126-144. Second edition.

O'Neal, M. E., Gray, M. E., and Smith, C. A. 1999. Population characteristics of a western corn rootworm (coleoptera: chrysomelidae) strain in east-central Illinois corn and soybean fields. J. Economic Entomol. 92(6):1301-1310.

Onstad, D. W., and Joselyn, M.G. 1999. Modeling the spread of western corn rootworm (coleoptera: chrysomelidae) populations adapting to soybean corn rotation. Environ. Entomol. 28(2):188-194.

Ostlie, K. R. 1987. Extended diapause of northern corn rootworm adapts to crop rotation. Crops Soils Magazine 39:23-25.

Payne, J., Fernandez-Cornejo, J., and Deberkow, S. 2003. Factors affecting the likelihood of corn rootworm Bt seed adoption. AgBioForum 6:79-86.

Pike, D. 1999. Environmental Toxicities and Properties of Common Herbicides. Chapter 18, pp. 309-313. *In:* 2000 Illinois Agricultural Pest Management Handbook. College of Agricultural, Consumer and Environmental Sciences, University of Illinois at Urbana-Champaign. University of Illinois Board of Trustees. ISBN 1-883097-25-8. Available at: *http://www.ag.uiuc.edu/%7Evista/abstracts/aiapm2k.html*

Privalle, L. 2002. Phosphomannose Isomerase, a Novel Plant Selection System. Annals of the New York Academy of Sciences 964:129-138.

Reidell, W.E. 1990. Rootworm and mechanical damage effects on root morphology and water relations in maize. Crop Sci. 30:628-631.

Sankula, S. and Blumenthal, E. 2004. Impacts on US Agriculture of Biotechnology-Derived Crops Planted in 2003 – An Update of Eleven Case Studies. National Center for Food and Agricultural Policy: www.ncfap.org.

Sánchez Gonzàlez, J. J. and Ruiz Corral, J.A. 1997. Teosinte Distribution in Mexico. *In:* Gene Flow Among Maize Landraces, Improved Maize Varieties, and Teosinte: Implications for Transgenic Maize. (eds. Serratos, J.A., Willcox, M.C., and Castillo-González, F.). pp. 18-39. Mexico, D.F., CIMMYT.

Spike, B.P. and Tollefson, J. J. 1991. Yield response of corn subjected to western corn rootworm (Coleoptera: Chrysomelidae) infestation and lodging. J. Econ. Entomol. 84:1585-1590.

Stewart Jr., C. N., Halfhill, M. D., and Warwick, S. I. 2003. Transgene introgression from genetically modified crops to their wild relatives. Nature Reviews Genetics 4:806-817.

Tollefson, J. J. 1988. A pest insect adapts to cultural control of crop rotations. *In*: Brighton Crop Protection conference. Pests and diseases, Volume 3, pp. 1029-1033.

USDA-ARS. 2003. Areawide Corn Rootworm Project – What works, what doesn't. This document is available at: http://www.areawiderootworm.info/final_report.pdf

USDA-NASS. 2004. Agricultural Chemical Usage 2003 Field Crops Summary. pp. 21-48. This document is available at: http://usda.mannlib.cornell.edu/reports/nassr/other/pcu-bb/agcs0504.pdf

USDA-NASS. 2000. Agricultural Chemical Usage 1999 Field Crops Summary. pp. 5-23. This document is available at: http://usda.mannlib.cornell.edu/reports/nassr/other/pcu-bb/agch0500.pdf

U.S. EPA. 2000. Issues pertaining to the Bt plant pesticides Risk and Benefit Assessments. The document is available at: http://www.epa.gov/scipoly/sap/2000/index.htm

U.S. EPA. 2001. *Bacillus thuringiensis* Cry3Bb1 and Cry2Ab2 protein and the genetic material necessary for its production in corn and cotton; exemption from the requirement of tolerance. 66 *FR* 24061-24066. The document is available at:
http://www.epa.gov/fedrgstr/EPA-PEST/2001/May/Day-11/p11917.htm

U.S. EPA. 2001. Biopesticide resgistration action document – Bacillus thuringiensis plant-incorporated protectants. The document is available at:
http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm

U.S. EPA. 2001. *Bt* Plant Incorporated Protectants October 15, 2001 Biopesticides Registration Action Document. The document is available at:
http://www.epa.gov/pesticides/biopesticides/pips/bt_brad2/2-id_health.pdf

Van Rie, J., Jansens, S., Hofte, H., Degheele, D., and Van Mellaert, H. 1989. Specificity of *Bacillus thuringiensis* ä-endotoxins, importance of specific receptors on the brush border membrane of the mid-gut of target insects. Eur. J. Biochem. 186:239-247.

Van Rie, J., Jansens, S., Hofte, H., Degheele, D., and Van Mellaert, H. 1990. Receptors on the brush border membrane of the insect midgut as determininants of the specificity of *Bacillus thruringiensis* delta-endotoxins. Appl. Environ. Microbiol. 56:1378-1385.

Wilkes, H. G. 1967. Teosinte: the closest relative of maize. Bussey Inst., Harvard Univ., Cambridge, Massachusetts.

Wilkes, H. G. 1977. Hybridization of maize and teosinte in Mexico and Guatemala and the improvement of maize. Econ. Bot. 31:254-293.

Wolfersberger, M.G., Hofmann, C., and Luthy, P. 1986. *In:* Bacterial Protein Toxins. (eds. Falmagne, P., Alout, J.E., Fehrenbach, F.J., Jeljaszewics, J. and Thelestam, M.) pp. 237-238. Fischer, New York.

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Appendix A. Potential for introgression from *Zea mays* to its sexually compatible relatives.

Wild diploid and tetraploid members of *Zea* collectively referred to as teosinte are normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua. A few isolated populations of annual and perennial teosinte have been reported to exist in Florida and Texas, respectively, but local botanists and agronomists familiar with the flora of these regions have not documented any current populations of teosinte (U.S. EPA, 2000). The Mexican and Central America teosinte populations primarily exist within and around cultivated maize fields; they are partially dependent on agricultural niches or open habitats, and in some cases are grazed upon or fed to cattle which distribute the seed. While some teosinte may be considered to be weeds in certain instances, they are also used by some farmers for breeding improved maize (Sánchez and Ruiz, 1997, and references therein).

All teosinte members can be crossed with cultivated corn to produce fertile F_1 hybrids (Doebley, 1990a; Wilkes, 1967; and Jesus Sánchez, personal communication, 1998). In areas of Mexico and Guatemala where teosinte and corn coexist, they have been reported to produce hybrids. Of the annual teosinte, *Z. mays* ssp *mexicana* forms frequent hybrids with maize, *Z. luxurians* hybridizes only rarely with maize, whereas populations of *Z. mays* ssp. *parviglumis* are variable in this regard (Wilkes, 1977; Doebley, 1990a). Fewer fertile hybrids are found between maize and the perennial *Z. perennis* than are found with *Z. diploperennis* (J. Sánchez, personal communication, 1998). Research on sympatric populations of maize and teosinte suggests introgression has occurred in the past, in particular from maize to *Z. mays* ssp. *luxurians* and *Z. mays* ssp. *diploperennis* and from annual Mexican plateau teosinte (*Z. mays* ssp. *mexicana*) to maize (Kato, 1997 and references therein). Nonetheless, in the wild, introgressive hybridization from maize to teosinte is currently limited, in part, by several factors including distribution, differing degrees of genetic incompatibility, differences in flowering time in some cases, block inheritance, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Doebley, 1990a; Galinat, 1988). First-generation hybrids are generally less fit for survival and dissemination in the wild, and show substantially reduced reproductive capacity which acts as a significant constraint on introgression. Teosinte has coexisted and co-evolved in close proximity to maize in the Americas over thousands of years, but maize and teosinte maintain distinct genetic constitutions despite sporadic introgression (Doebley, 1990a).

The genus *Tripsacum* contains up to 16 recognized species, most of which are native to Mexico, Central and South America. But three *Tripsacum* species, *T. floridanum*, *T. lanceolatium*, and *T. dactyloides*, exist as wild and/or cultivated in the U.S. (Hitchcock, 1971). Though many of these species occur where corn might be cultivated, gene introgression from line 1507 corn under natural conditions is highly unlikely or impossible. Hybrids of *Tripsacum* species with *Zea* are difficult to obtain outside of a laboratory and are often sterile or have greatly reduced fertility, and none are able to withstand even the mildest winters (Beadle, 1980; Galinat, 1988).

References (see EA, Literature Cited, Section VII.)

Appendix B. **Environmental and human health safety of mCry3A (as expressed in corn line MIR604 or as purified from a microbial source) compared to other common insecticides used on corn to control the corn rootworm target pests, and other non-target pests.**

	mCry3A	Terbufos (Counter®)	Tefluthrin (Force®)
Environmental Fate	The DT_{50} estimate for mCry3A protein in soil was found to be 7.6 days. (1)	Terbufos hydrolyzes at pH 5, 7, and 9 with a half-life of 2.2 weeks. Formaldehyde was the major degradate detected in this study. Aerobic soil metabolism study indicate that terbufos degrades in silt loam soil with a half-life of 26.7 days. The major degradates detected in this study included carbon dioxide, terbufos sulfoxide, and terbufos sulfone. Terbufos residues have a half-life of less than 40 days in field plots of loam soil treated with a 15 percent granular formulation at an application rate of 1 lb ai/A. The sampling protocol was inadequate to accurately assess the dissipation of terbufos residues in field soil and a new study is required. The available data reviewed by the Agency are not sufficient to fulfill data requirements nor to assess the environmental fate of terbufos. EPA is concerned about the potential for the two degradates, terbufos sulfoxide and sulfone, to leach to groundwater, and the potential for parent terbufos and the sulfoxide and sulfone degradates to runoff to surface water. Terbufos parent degrades rapidly to the sulfoxide and sulfone metabolites, and is considered moderately mobile. Terbufos sulfoxide and sulfone are more mobile and persistent than parent terbufos. The acute DWLOCs calculated for the general U.S. population is 8.1 Fg/L. The chronic DWLOCs calculated for the general U.S. population is 1.7 Fg/L. Maximum acute and chronic estimated environmental concentrations (EECs) for	Tefluthrin is immobile in soil and, therefore, will not leach into ground water. Additionally, due to the insolubility and lipophilic nature of tefluthrin, any residues in surface water will rapidly and tightly bind to soil particles and remain with sediment, therefore not contributing to potential Tefluthrin is immobile in soil and, therefore, will not leach into ground water. Additionally, due to the insolubility and lipophilic nature of tefluthrin, any residues in surface water will rapidly and tightly bind to soil particles and remain with sediment, therefore not contributing to potential dietary exposure from drinking water. Plant metabolism studies indicate that tefluthrin per se is not translocated to plants but is degraded in soil to two principal metabolites that are capable of being taken up by plants. EPA has decided that Metabolite VI need not be regulated. Based on tefluthrin not being registered for residentia1 non-food sites, EPA concludes that the aggregate short- and intermediate-term risks do not exceed levels of concern (MOE 1ess than 100), and that there is reasonable certainty that no harm will result from aggregate exposure to tefluthrin residues. *(5*)

		parent terbufos plus the sulfoxide and sulfone degradates exceed the acute and chronic DWLOCs, respectively, in all cases. *(2)*	
Avian toxicity	Feeding mCry3A plant material to broiler chickens supported growth and mortality rates that were not significantly different than that supported by its isogenic controls (1) Feeding mCry3A grain from event MIR604 to Northern Bobwhite resulted in no adverse effects on mortality, weight gain, and feed consumption. (1) LD_{50} mCry3A > 652 mg protein/ kg body weight	Seven incidents to nontarget terrestrial organisms have been reported. Up to three of the incidents had some indication of misuse or misapplication. All the mortalities involved bird species (mostly raptors), with the exception of one incident involving red wolves in North Carolina, which is believed to be the result of an intentional poisoning. Calculated RQs for birds and mammals significantly exceed EPA's risk concern for both granular formulations. *(2)* Dietary Avian Toxicity: 143 and 157 ppm (from two bobwhite studies). - Avian Reproduction: Terbufos was not considered to produce avian reproductive effects based on results of a bobwhite quail study and a mallard duck study. *(3)*	Low toxicity to birds *(6)*.
Fish toxicity	Feed prepared using plant-produced mCry3A protein to rainbow trout resulted in no adverse effects. (1) Exposure rate = 37.0X EEC	EPA has concerns about risk to nontarget aquatic organisms from parent terbufos and the terbufos sulfoxide and sulfone degradates based on widespread fish kill incidents involving terbufos use on corn with all application methods. These concerns are further supported by standard LOC criteria, which indicate risk concerns to aquatic fish and invertebrates associated with both the clay-based (15% active ingredient) and polymer-based (20% active ingredient) granular formulations using banded applications.*(2)* Terbufos ranks	Highly toxic to fish *(6)*

		fourth in pesticide-induced fish kills reported to the Agency, and is the leading cause of fish kills from use on corn. **Freshwater Fish Acute Toxicity**: Ranges from 0.77 to 20.00 ppb. - Freshwater Invertebrate Acute Toxicity: 0.31 ppb for Daphnia magna. - Marine/Estuarine Fish Acute Toxicity: Data gap.- Marine/Estuarine Invertebrate Toxicity: Data gap. Mollusk toxicity: Data gap *(2)*	
Nontarget and beneficial organisms	mCry3A microbially produced protein were fed to non-target insects and resulted in no adverse effects. (1) **Predatory arthropod, flower bug:** LC_{50} >50 µg mCry3A/g diet; NOEC = 50 µg mCry3A/g diet; Exposure rate ≥ 10.6X EEC (1) **Lady beetle:** LC_{50} >50 µg mCry3A/mL; NOEC = 50 µg mCry3A/mL; Exposure rate ≥ 12.3X EEC (1) **Rove beetle**: **:** LC_{50} >50 µg mCry3A/mL; NOEC = 50 µg mCry3A/mL; Exposure rate ≥ 15.6X EEC (1) **Ground beetle**: **:** LC_{50} >50 µg mCry3A/g blowfly pupa;	Terrestrial Field Study (Level 1): both soil-incorporated (2 lb ai/A) and nonsoil-incorporated (1 lb/A) resulted in nontarget mortalities, with the latter application much more severe in its effects *(2*,6)	Data not found.

	NOEC = 50 µg mCry3A/g blowfly pupa; Exposure rate ≥ 11.2X EEC (1)		
Honey bee toxicity	**Larval honey bees** were fed microbially-produced mCry3A in a sucrose solution. (1) LC_{50} >50 µg mCry3A/g solution; NOEC = 50 µg mCry3A/g solution; Exposure rate ≥ 35.7X EEC (1)	Not described in available studies.	High toxicity to bees *(7)*
Mammalian toxicity	A single dose of mCry3A microbially produced protein was fed to mice and no acute oral toxicity or adverse effects in terms of body weight, detailed clinical observations and gross-pathological lesions were observed. (1) LD_{50} > 2377 mg mCry3A/kg body weight; NOEC = 2377 mg mCry3A/kg body weight; Exposure rate ≥ 2600X EEC (1)	**Acute Oral**: Toxicity Category I (1.6 and 1.3 mg/kg for male and female rats, respectively). - **Acute Dermal**: Toxicity Category I (0.81 and 0.93 mg/kg for male and female rabbits, respectively). - **Acute Inhalation**: Toxicity Category I (< 0.2 mg/L). - **Delayed Neurotoxicity**: No evidence of acute delayed neurotoxicity at the 40 mg/kg dosage level tested in hens. - **Subchronic Feeding**: The NOEL for both systemic effects and cholinesterase inhibition in a rat subchronic study is 0.25 ppm. - **Subchronic Dermal**: The NOEL for systemic effects in a 30-day rabbit study is 0.020 mg/kg. - **Mutagenicity**: Terbufos did not exhibit mutagenic potential in the Ames assay, the in vivo cytogenetic assay, and the dominant lethal test.	**Acute toxicity** studies with the technical grade of the active ingredient tefluthrin: oral LD50 in the rat is 21.8 mg/kg for males and 34.6 mg/kg for females; dermal LD50 in the rat is 316 mg/kg in males and 177 mg/kg in females; acute inhalation LC50 in the rat is 0.037 mg/l and 0.049 mg/l in male and female rats, respectively; primary dermal irritation study in the rabbit showed slight irritation; and the acute delayed neurotoxicity study did not show acute delayed neurotoxicity. In an oral toxicity study, the NOEL for female rats is 100 ppm (equivalent to approximately 5 mg/kg/day). The NOEL for skin effects in rats is 1.0 mg/kg). The NOEL for neurological effects (the observed postural effects) may be between 0.025 and 0.1 mg/kg. **Carcinogenicity**: There was no evidence of carcinogenic potential. **Mutagenicity**: There is no mutagenicity concern. **Metabolism**: In both rats and dogs, when given either 1 or 10 mg/kg, most of the radioactivity was found

		- **Teratogenicity**: The NOEL for developmental toxicity in a rat teratology study is 0.1 mg/kg/day. - **Reproduction**: The NOEL for reproductive effects in a three-generation rat reproduction study is 0.25 ppm. - **Oncogenicity**: None *(2*,6)	in the feces unchanged and most urinary metabolites were conjugated. In rats, the halflife in the liver is 4.8 days, in the fat is 13.3 days and in the blood is 10.6 days. In a study with rat fat, half of the radioactive residues could be attributed to the parent and the remaining residues consisted of a mixture of fatty acid esters of hydroxylated parent metabolites. **Neurotoxicity**: No acceptable mammalian neurotoxicity studies *(5*). are available.*(5*)
Nontarget soil organism effects	Earthworms were exposed to soil containing microbially produced mCry3A protein and no adverse effects were observed. (1) **Earthworms** LC_{50} >250 µg mCry3A/g moistened soil; NOEC = 250 µg mCry3A/g moistened soil; Exposure rate ≥ 46X EEC (1)	Not described by present reports.	Not found in these reports
Toxicity	Not assigned	Classified by EPA as Toxicity Category I	Toxicity class I for dermal, oral, inhalation exposures, and Class IV for skin irritation.
EDF's Integrated Environmental Rankings - Combined human & ecological scores *(4*)	Not ranked	85-100% where 0 is the lowest and 100 is the highest hazard rating *(4*).	Data lacking; not ranked by any system in Scorecard.

Abbreviations: LD_{50} = Nominal Median Lethal Dose ; LC_{50} = Nominal Median Lethal Concentration; EEC = Estimated Environmental Concentration; NOEC = No Observable Effect Concentration

Sources of information:

1. Petition for Determination of Non-regulated Status: Corn Rootworm Protected Transformation Event MIR604 – Revised.

2. Overview of Revised Terbufos Risk Assessment, Office of Pesticide Programs-- US EPA, http://www.epa.gov/pesticides/op/terbufos/terbufosview.htm
3. EPA Pesticide Fact Sheet http://www.epa.gov/REDs/factsheets/terbufos_ired_fs.htm
4. Environmental Defense Fund Scorecard. http://www.scorecard.org/chemical-profiles/
5. Tefluthrin; Pesticide Tolerance ENVIRONMENTAL PROTECTION AGENCY (40 CFR Part 180) [Federal Register: November 26, 1997 (Volume 62, Number 228) http://www.epa.gov/EPA-PEST/1997/November/Day-26/p30946.htm
6. Farm Chemicals Handbook, Meister Publishing, p. C374.
7. Ohio State University, Insect Pests of Field Crops Bulletin 545 Toxicity of Pesticides http://netc2000.tamu.edu/abstracts/tx009/paper/~ohioline/b545/index.html

Determination of nonregulated status for corn line MIR604

In response to the petition 04-362-01p from Syngenta Seeds, Inc., APHIS has determined that corn line MIR604 and progeny derived from it are no longer regulated articles under APHIS regulations at 7 CFR Part 340. Permits or acknowledged notifications that were previously required for environmental release, importation, or interstate movement under those regulations will no longer be required for MIR604 corn and its progeny. Importation of seeds and other propagative material would still be subject to APHIS foreign quarantine notices at 7 CFR Part 319 and the Federal Seed Act regulations at 7 CFR Part 201. This determination is based on APHIS' analysis of field, greenhouse and laboratory data, references provided in the petition, and other relevant information as described in this environmental assessment that indicate that MIR604 poses no more of a plant pest risk than its non-genetically engineered counterpart. The transgenic event found in MIR604 will not pose a plant pest risk for the following reasons: (1) gene introgression from MIR604 corn into wild relatives in the United States and its territories is extremely unlikely and is not likely to increase the weediness potential of any resulting progeny nor adversely affect genetic diversity of related plants any more than would introgression from traditional corn hybrids; (2) it exhibits no characteristics that would cause it be weedier than the non-genetically engineered parent corn line or other cultivated corn; (3) it does not pose a risk to non-target organisms, including beneficial organisms and threatened or endangered species, because the insecticidal activity of the mCry3A protein is limited to target pest species, namely corn rootworm; (4) it does not pose a threat to biodiversity as it does not exhibit traits that increase its weediness and its unconfined cultivation should not lead to increased weediness of other cultivated corn, it exhibits no changes in disease susceptibility, and it is unlikely to harm non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service; (5) compared to current corn pest and weed management practices, cultivation of MIR604 corn should not impact standard agricultural practices in corn cultivation and controlling volunteer corn, including those for organic farmers; and (6) disease susceptibility and compositional profiles of MIR604 corn are similar to those of its parent variety and other corn cultivars grown in the United States, therefore no direct or indirect plant pest effects on raw or processed plant commodities are expected.

In addition to our finding of no plant pest risk, there will be no effect on the threatened or endangered species resulting from a determination of nonregulated status for MIR604 and its progeny. APHIS also concludes that new varieties bred from MIR604 corn are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any observed for corn line MIR604, or those observed for other corn varieties not considered regulated articles under 7 CFR Part 340.

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