USDA/APHIS Draft Environmental Assessment

In response to Monsanto Petition 06-298-01p seeking a
Determination of Non-regulated Status for
Bt Cry1A.105 and Cry2Ab2 Insect Resistant Corn Event
MON 89034

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 an Environmental Assessment (EA) in response to a petition (APHIS Number 06-298-01p) from Monsanto Company (hereafter referred to as Monsanto) regarding the regulatory status of genetically engineered (transformed) European corn borer resistant corn derived from transformation event MON 89034. 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 MON 89034 corn have been conducted under permits issued or notifications acknowledged by APHIS. Monsanto petitioned APHIS requesting a determination that MON 89034 corn is not a plant pest, and therefore MON 89034 corn and progeny derived from crosses with other non-regulated corn should no longer be regulated articles under these APHIS biotechnology regulations.

II. Introduction

Monsanto has submitted a "Petition for Determination of Non-regulated Status" to the USDA/APHIS (APHIS number 06-298-01p) for genetically engineered corn plants, event line MON 89034 (hereafter referred to as MON 89034), that are resistant to the feeding damage caused by the European corn borer (ECB; *Ostrinia nubilalis*). In addition to controlling ECB (the primary target pest), MON 89034 provides greater protection against lepidopteran pests such as the Asian corn borer (ACB; *Ostrinia furnacalis*), southwestern corn borer (SWCB; *Diatraea grandiosella*), sugarcane borer (SCB; *Diatraea saccharalis*), fall armyworm (FAW; *Spodoptera frugiperda*) and corn earworm (CEW; *Helicoverpa zea*) than previously deregulated corn containing insecticidal proteins such as MON 810.

Larvae of ECB, ACB, SWCB, SCB, and FAW feed and burrow on corn leaves, stem whorls, stalks and/or ears resulting in stalk lodging, dropped ears, and damaged grain. CEW feed primarily on the corn silk and ears resulting in yield loss and grain damage. *Bacillus thuringiensis* (Bt) bacteria produce a group of related toxins (delta endotoxins) that cause mortality when ingested by susceptible insects. Preparations of *B. thuringiensis* containing delta-endotoxins are used as foliarly applied biopesticides. However, they are not routinely effective against ECB and the other stalk boring larvae because, at certain stages, these larvae primarily feed inside the plants where the foliar applied biopesticide cannot reach. The same problem is encountered with other non-systemic, foliarly applied chemical insecticides. The development and approval of transgenic corn plants expressing Bt delta-endotoxins has provided growers with another safe and efficacious option for the control of ECB which growers have widely embraced.

Monsanto produced MON 89034 corn by *Agrobacterium*-mediated transformation of corn with PV-ZMIR245, a binary vector containing two T-DNAs. The first T-DNA contains the *cry1A.105* and *cry2Ab2* expression cassettes. The second T-DNA contains the *nptII* (neomycin phosphotransferase II) expression cassette. The *nptII* gene was used as a selectable marker and was eliminated by traditional breeding methods in the later stages of development of MON 89034. Thus, MON 89034 contains only the *cry1A.105* and *cry2Ab2* expression cassettes.

MON 89034 corn has been field tested in a wide variety of locations (24 States and Puerto Rico) since 2001 under notifications from APHIS that are listed in the amended Table H-1 found on page 11 of the addendum to the petition dated January 23, 2007. This field testing was conducted, in part, to confirm that MON 89034 corn exhibits the desired agronomic characteristics and does not pose plant pest or environmental risks. Field tests conducted under APHIS oversight allow for evaluation in an 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 or environmental risk.

A. USDA Regulatory Authority

APHIS regulations at 7 CFR part 340, which were promulgated pursuant to 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 be a plant pest. 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. A plant pest is an organism that poses a direct or indirect risk to other plants or plant products. The term plant pest generally refers to insects and diseases. But in the case of USDA biotechnology regulation, it refers to GE organisms that have the potential to be plant pests. The resulting GE organisms are not necessarily plant pests. However, APHIS' evaluation and review process to determine whether a GE organism is a plant pest has to be performed and completed before a GE organism can be no longer considered a regulated plant pest. APHIS regulations provide a list of the organisms regarded as plant pests so applicants know if the organism they are developing is a plant pest. If the organism being engineered is on the list, then the engineered product will be considered to be a potential plant pest until determined otherwise. If DNA from any organism on the list was used to produce the GE organism, the GE organism will be regarded as a potential plant pest even if the parental organism is not.

Two common examples of APHIS' regulations being invoked due to use of DNA in the engineering process are plants that contain small fragments of DNA from plant pests to control expression of new traits; and plants which contain DNA from specific plant viruses that cause the plant to be resistant to that same virus. Even if the original organism itself is not on the list and no DNA sequences from organisms on the list were used in the engineering, it still may be subject to APHIS biotechnology regulations if it is an unclassified organism or if there is reason to believe that the resulting GE organism is or could be a plant pest. MON 89034 corn has been considered a regulated article because it was genetically engineered with regulatory sequences derived from bacterial and plant virus plant pests.

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 is not a plant pest, and therefore should no longer be regulated. If

APHIS determines that the regulated article is not a plant pest, 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.

MON 89034 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 insecticides, 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.

On July 17, 2006, the EPA announced a temporary exemption from the requirement of a tolerance for residues of the *Bacillus thuringiensis* Cry1A.105 protein and the genetic material necessary for its production in corn on field corn, sweet corn, and popcorn when applied/used as a plant-incorporated protectant (PIP). The temporary tolerance exemption will expire on June 30, 2009 (71 *FR* 40427-40431). On July 17, 2006 EPA announced a temporary exemption from the requirement of a tolerance for residues of the *Bacillus thuringiensis* Cry2Ab2 protein and the genetic material necessary for its production in corn on field corn, sweet corn, and popcorn when applied/used as a PIP. The temporary tolerance exemption will expire on June 30, 2009 (71 *FR* 40431-40436). Pursuant to its authority under the FFDCA, EPA conducted a comprehensive assessment of the Cry1A.105 and Cry2Ab2 proteins and the genetic material necessary for their production in corn, including a review of acute oral toxicity data on the Cry1A.105 and Cry2Ab2 proteins, amino acid sequence comparisons to known toxins and allergens, as well as data demonstrating that the Cry1A.105 and Cry2Ab2 proteins are rapidly degraded by gastric fluid in vitro, are not glycosylated, and are present in low levels in corn tissue. Based on their assessment, EPA concluded that there is a reasonable certainty that no harm will result from dietary exposure to this protein as expressed in genetically modified corn. On January 24, 2007, the EPA announced the issuance of an Experimental Use Permit (534-EUP-97) to Monsanto for the use of 165,700 lbs of MON 89034 corn seed containing 0.47 lbs of the *Bacillus thuringiensis* Cry1A.105 protein and the genetic material necessary for its production (vector PV-ZMIR245) and 0.41 lbs of the *Bacillus thuringiensis* Cry2Ab2 protein and the genetic material necessary for its production (vector PV-ZMIR245) in corn (72 *FR* 3133-3135).

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. Monsanto submitted a summary of their safety assessment to the FDA on October 13, 2006. The consultation for MON 89034 corn as food and feed is currently underway.

III. Purpose and Need

APHIS prepared this draft environmental assessment (EA) as the environmental review and analysis part of its regulatory determination on the status of MON 89034 corn as regulated articles under APHIS regulations. Monsanto submitted a petition to USDA-APHIS requesting that APHIS make a determination that this corn should 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 nonregulated 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 continue to determine that MON 89034 corn and its progeny is a regulated article and should continue to be regulated under 7 CFR Part 340. Therefore, permits or acknowledgment of notifications from APHIS would still be required for introduction of MON 89034 or its progeny. APHIS would choose this alternative if there were insufficient evidence to determine that MON 89034 is not a plant pest and could not be deregulated.

B. Determination of Nonregulated Status

Under this alternative, MON 89034 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 MON 89034 corn or its progeny. A basis for this determination would be a finding that MON 89034 is not a plant pest based on information submitted in the petition as stipulated in 7 CFR Part 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. Preferred Alternative

For the reasons discussed below, APHIS has chosen Alternative B as the preferred alternative. This is based on the lack of plant pest characteristics in MON 89034 corn. Data submitted by Monsanto and reviewed by APHIS in addition to published research (e.g., Pilcher *et al*. 1997, Venditti and Steffey 2002, Jasinski *et al*. 2003, Daly and Buntin 2005, Dively 2005, Pilcher *et al*. 2005, Marvier *et al*. 2007) verified that MON 89034 will not pose a direct or indirect risk to other plants or plant products.

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 Cry1A.105 and Cry2Ab2 proteins in MON 89034 corn is not expected to alter the range of corn cultivation within the United States.

B. Susceptible lepidopteran corn pests

Bt is a naturally occurring, Gram-positive bacterium found in many environments including soil, insects, stored-product dust, and deciduous and coniferous leaves. Crystal proteins, called Cry toxins or delta-endotoxins, form within the spores of Bt bacteria. When ingested by a susceptible insect, these proteins readily bind to receptors on the midgut, insert into its membrane (Gill, Cowles, and Pietrantonio, 1992; Schnepf et al., 1998), and form pores causing destruction of cells, leading to starvation, gut paralysis, septicemia (blood poisoning), and death of the insect (Schnepf et al., 1998).

Lepidopteran pest larvae feed on leaves, stalks, silks and ears of corn and can significantly affect corn yields. Feeding by borers such as ECB, ACB, SWCB, and SB larvae cause direct and indirect damage to corn. Of these Lepidoptera, ECB is the predominant pest of corn in the U.S. Early instar larvae feed in the whorl and on leaf tissue of corn plants resulting in a buckshot appearance or window-paning, respectively. Late instar larvae burrow into the midribs of leaves, causing them to break, or into stalks of corn plants, causing lodging and increased susceptibility to stalk rot and other diseases. Larvae also feed in the sheath, collar tissue, and leaf axils of the ear zone in older corn plants. Feeding on kernels may cause shank tunneling and result in dropped ears. (http://www.mda.state.mn.us/pestsurvey/Factsheets/ecb.html accessed on February 6, 2007).

Corn earworm feed on foliage, silks and ears of corn. Direct feeding on silks prior to pollination may result in sub-optimal kernel-fill kernels. Direct feeding on kernels is the major cause of economic damage by CEW and may also cause secondary infections which produce toxins (http://www.mda.state.mn.us/pestsurvey/Factsheets/cornearworm.html accessed on February 6, 2007). Fall armyworm feeding on leaf tissue often results in only the midrib remaining. (http://www.lgseeds.com/LG_Tech2/fallarmyworm.asp accessed on February 6, 2007).

C. Genetically engineered insect-resistant corn

The toxicity of Bt Cry1 and Cry2 proteins to certain Lepidoptera is a well known and a widely published phenomenon. The introduction of Bt corn products has provided growers with an effective means to control stalk-boring and other lepidopteran pests. A discussion of potential impacts of Bt corn on the human environment appears below and additional information is in Appendix A of this EA.

VI. Potential Environmental Impacts

Potential impacts to be addressed in this EA are those that pertain to the use of MON 89034 corn and its progeny in the absence of confinement.

A. Potential impacts from gene introgression from MON 89034 corn into its sexually compatible wild relatives.

In assessing the risk of gene introgression from MON 89034 corn into its sexually compatible wild 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 MON 89034 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 been occurring 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 American 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, 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 MON 89034 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 MON 89034 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 MON 89034 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 the *cry1A.105* or *cry2Ab2* pesticidal genes would not be expected to transform them into weeds.

B. Potential impacts based on the relative weediness of MON 89034 corn

APHIS reviewed data submitted by Monsanto in the petition and appendices and concurred that MON 89034 corn is not any more likely to become a weed than the non-transgenic recipient corn line, or other corn currently cultivated. The Monsanto assessment encompasses a thorough consideration of the basic biology of corn and an evaluation of unique characteristics of MON 89034 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).

Monsanto collected agronomic data from 18 field test sites in 2004 and 2005 to assess 14 plant growth and development characteristics, five seed germination parameters, two pollen characteristics, and more than 70 observations for each of the plant-insect, plant-disease and plant responses to abiotic stressor interactions. Table VII-1 (page 113 of the petition) lists the agronomic characteristics evaluated during field tests. Results indicate no viable hard seed in any of the temperatures tested and no statistical differences in germination between MON 89034 and conventional control corn (Table VII-3, page 119 of the petition). There were also no statistical differences between MON 89034 and conventional corn detected for pollen diameter or viability (Table VII-4, page 120 of the petition), seedling vigor, early stand count, final stand count, days to 50% pollen shed, days to 50% silking, stay green, ear height, dropped ears, root lodged plants, grain moisture, test weight and yield (Tables VII-6, VII-7 and VII-8, pages 126 - 128 of the petition). Although plant height was slightly lower and the number of stalk-lodged plants were less for MON 89034 than conventional corn in 2004, these differences were not detected in 2005 (Table VII-6, page 126 of the petition). Since the mean values of MON 89034 fell well within the ranges of values observed for the 23 reference commercial corn hybrids grown beside MON 89034, differences observed in 2004 are not likely to contribute to increased plant pest potential. In addition, no repeatable differences in biotic (e.g., insects and diseases) and abiotic (e.g., drought, wind, nutrient deficiency) stressors were observed in 255 comparative observations of MON 89034 and conventional corn (Tables VII-9, VII-10 and VII-11, pages 131 – 141 of the petition).

The introduced lepidopteran insect resistance traits are not expected to cause MON 89034 corn to become a weed because corn has many characteristics that prevent it from persisting in the environment without human intervention. Other lepidopteran-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 MON 89024 corn would release it from any constraint that would result in increased weediness. MON 89034 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 MON 89034 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.

Research has established the specificity of the Cry1 and Cry2 class of insecticidal proteins 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 lepidopteran specific Cry proteins is 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 birds, mammals, and humans (Shimada et al., 2005; Shimada et al., 2006; 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, Sacchi et al., 1986).

Potential impacts on target and non-target pests:

Target pests of the Cry1A.105 and Cry2Ab2 proteins expressed in MON 89034 corn include the larvae of certain Lepidoptera. MON 89034 provides enhanced benefits for the control of lepidopteran insect pests relative to MON 810 which contains the Cry1Ab protein. MON 810 was granted a determination of non-regulated status on March 15, 1996 (61 *FR* 10720, http://www.aphis.usda.gov/brs/aphisdocs2/96_01701p_com.pdf).

Laboratory tests were conducted to evaluate the spectra of activity of the Cry1A.105 and Cry2Ab2 proteins on representative corn pests from three insect orders including four Lepidoptera (ECB, CEW, FAW and black cutworm (BCW; *Agrostis ipsilon*)), two Coleoptera (boll weevil (*Anthonomus grandis grandis*) and southern corn rootworm (*Diabrotica undecimpunctata howardi*)), and two Hemiptera (western tarnished plant bug (*Lygus Hesperus*) and green peach aphid (*Myzus persicae*)). These tests indicated that Cry1A.105 and Cry2Ab2 are not active against the coleopteran and hemipteran species evaluated and are active against the target lepidopteran insect pests.

Field efficacy trials conducted in multiple locations in the U.S. and Puerto Rico during 2004 and 2005 demonstrated a high level of efficacy of MON 89034 against several lepidopteran insect pests. Efficacy of MON 89034 against ECB, SWCB and SCB was equivalent to MON 810 and MON 89034 provided superior control of FAW and CEW. MON 89034 also provided some protection from BCW and western bean cutworm (WBC; *Striacosta albicosta*), but additional testing is needed to confirm this activity.

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

Potential changes in toxicity and allergenicity of Bt proteins to mammal, avian, and aquatic organisms are described in Appendix A of this EA. The Cry1A.105 and Cry2Ab2 proteins are 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 MON 89034 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 conducted and submitted by Monsanto that were designed to evaluate the sensitivity of representative non-target organisms to Cry1A.105 and Cry2Ab2 proteins. Test substrates included corn plant material (e.g., lyophilized corn leaf tissue, pollen, or grain) expressing Cry1A.105 and/or Cry2Ab2 proteins or protein purified from *E. coli* engineered to express the Cry 1A.105 or Cry2Ab2 protein. Monsanto verified that the bacterially-produced Cry1A.105 and Cry2Ab2 proteins were engineered to match the amino acid sequences of their counterparts expressed in MON 89034 corn. Physicochemical and functional equivalence of the bacterially-produced proteins to MON 89034-produced proteins were examined to verify the validity of using the *E. coli*-produced proteins as appropriate surrogates. Characterization and equivalence of the proteins were confirmed from Western blot analysis, SDS-PAGE, MALDI-TOF MS, N-terminal sequence analysis, glycosylation analysis, and insect activity bioassay. Since the Cry1A.105 and Cry2Ab2 proteins do not share any amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins, MON 89034 are not expected to cause adverse effects to mammals.

A summary of the studies evaluating potential impacts on non-target organisms are summarized in the Addendum to Monsanto's petition dated January 23, 2007. Non-target organisms were dosed with at least 10 times the maximum expected environmental concentrations (MEEC) of Cry1A.105 and Cry2Ab2 proteins present in the plant tissue most likely to be ingested. The source and estimates of the margins of exposure (MOE) of non-target arthropods to the Cry1A.105 and Cry2Ab2 proteins are listed in Table VIII-2 (page 157) of the petition. Tests included acute dietary toxicity studies with the following representative beneficial arthropods: 1. Soil-dwelling organisms, including Collembola (*Folsomia candida*) and earthworms (*Eisenia fatida*); 2. Aquatic organisms including *Daphnia magna* (water flea); 3. Avian species including the bobwhite quail (*Colinus virginianus*); 3. Honey bees (*Apis mellifera*), an important pollinator; and 4. Biological control organisms including (a) minute pirate bug (*Orius insidiosus*; aka insidious flower bug), (b) lady beetle (*Coleomegilla maculata*), and (c) an Ichneumonid parasitic wasp (*Ichneumon promissorius*). Results of these studies indicate that no effects on mortality or reproduction on these organisms would be expected due to expected routes of exposure or feeding on MON 89034 corn. This analysis took into consideration the levels of the Cry1A.105 and Cry2Ab2 proteins measured in different tissues of MON 89034 corn, the environmental fate and likely routes and levels of exposure to the proteins, corn plant tissue or residues of this tissue that contain the active toxin, and dietary preferences. Results of the non-target organism studies are summarized in Table VIII-1 (page 156) of the petition.

Potential impacts on threatened and endangered arthropods:

Given the specificity of activity of the Cry1A and Cry2A proteins, species outside the insect order Lepidoptera should not be affected. APHIS has thoroughly examined all threatened and endangered lepidopterans that occur in counties where corn is grown, and determined that the breeding habitat of lepidopterans does not overlap corn (see Appendix B of this EA). Threatened endangered lepidopterans in the U.S. have very restrictive habitat ranges; and their larvae typically feed on specific host plants, none of which include corn or its sexually compatible relatives. An examination of county distribution of endangered lepidopterans shows that they do not occur in agricultural settings where corn is grown except the Karner blue butterfly (*Lycaeides Melissa samuelis*).

The Karner blue requires wild lupine *(Lupinus perennis)* as an oviposition substrate and larval food source, while the adults feed on wild flowers. As of 1992, Karner blue is known to exist along the northern extent of the range of wild lupine, where there are prolonged periods of winter snowpack, in parts of Wisconsin, Michigan, Minnesota, Indiana, New Hampshire, New York, and Illinois (Haack, 1993). Karner blue is associated with wild lupine growing on dry, sandy soils in pine-barrens, oak savannah, forest trails and previously disturbed habitats such as utility rights-of-way, military installations, airports, highway corridors, sand roads and sand pits, and abandoned farm fields (Haack, 1993). Wild lupine thrives in full sun to partial shade, and does not survive long in full shade (Haack, 1993), and thus would not survive long in a mature corn field. Likewise, the Karner blue is associated with areas of low to semi-closed canopy cover (Haack, 1993). Although there are two counties in Wisconsin that have been identified as having a potential overlap between corn pollen shed and the presence of Karner blue larvae, there is no evidence of Karner blue exposure to corn pollen in these locations. In addition, Monsanto conducted a risk analysis of MON 89034 corn pollen to the Karner blue butterfly that showed a 12-fold margin of safety in the event of the highest possible exposure to corn pollen. It is therefore unlikely Karner blue butterflies will be adversely affected by MON 89034 corn.

BRS has reviewed the data provided by Monsanto and available in scientific literature and available on distribution of species maintained on the Fish and Wildlife Service's website (http://www.fws.gov/endangered/wildlife.html#Species) to reach a determination that the release following a determination of non-regulated 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.

Environmental fate in soil:

A sensitive insect bioassay using the CEW was conducted in the laboratory to evaluate aerobic degradation of the Cry1A.105 and Cry2Ab2 proteins in soil. The DT_{50} for the Cry1A.105 protein ranged from 2 to 7 days and 0.5 to 3 days for Cry2Ab2. The DT_{90} for the Cry1A.105 protein ranged from 7 to 19 days and 3 to 13 days for Cry2Ab2. These data indicate that these proteins are degraded rapidly in soil so bioaccumulation of these Cry proteins in soil will not occur.

D. Potential impacts on biodiversity

APHIS has concluded that MON 89034 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. Since corn is not weedy and MON 89034 corn is no weedier than other corn, there is no effect on biodiversity. Evidence of lack of accumulation of Bt Cry proteins in the soil and lack of toxicity to non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service lead APHIS to conclude a lack of effect on 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 apparent potential for impact on biodiversity.

E. Potential impacts on agricultural and cultivation practices

APHIS considered potential impacts associated with the cultivation of lepidopteran-resistant corn MON 89034 on current agricultural practices, in particular, those used to control lepidopteran pests in corn. The potential impact on organic farming was also considered.

Current agricultural practices are themselves impacting various aspects of environmental and human health. Planting MON 89034 corn is not expected to adversely affect current cultivation and management practices for corn. Monsanto has provided data to demonstrate that there are no phenotypic, ecological, compositional or agronomic differences between MON 89034 and conventional corn. In addition, there is not an increased risk of disease in MON 89034 corn and it will not cause adverse effects to non-target arthropods compared to conventional corn. On the contrary, the most common mycotoxin found in corn grain, Fumonisin produced by a general class of fungi called Fusarium, has been shown to decrease in lepidopteran-active Bt corn (Munkvold and Hellmich 1999, Munkvold *et al*. 1997, Munkvold *et al*. 1999, Dowd *et al*. 2000). There will also be less disruption of non-target arthropod communities in Bt crops utilizing less insecticides than conventionally grown corn (e.g., Pilcher *et al*. 1997, Venditti and Steffey 2002, Jasinski *et al*. 2003, Daly and Buntin 2005, Dively 2005, Pilcher *et al*. 2005, Marvier *et al*. 2007).

Potential impacts of MON 89034 corn on insect control practices

The major pest controlled by lepidopteran-resistant Bt corn varieties is the ECB, but other important pests controlled to varying degrees are CEW, SWCB, and other stalk boring lepidopteran larvae. Commercialization of Bt crops has resulted in fewer insecticide applications and thus, lower management costs (Fitt, 2000; Schnepf et al., 1998; Sankula et al., 2005). Also, one notable advantage of GE insecticidal crops over conventional insecticides is the high specificity of the Bt toxins, which minimizes potential toxic effects on nontarget insects (Betz, Hammond, and Fuchs, 2000; Macintosh et al., 1990). Bt crops may also reduce the need for synthetic insecticides which, in turn, would decrease risks to the environment and effects on nontarget organisms, including beneficial insects.

MON 89034 was developed by Monsanto as a second generation insect protection corn product that provides enhanced benefits for the control of lepidopteran insect pests relative to MON 810. There has been a great amount of experience and familiarity with MON 810 since its commercial availability in 1996. The Cry1A.105 and Cry2Ab2 proteins expressed in MON 89034 corn will better serve corn growers' needs for controlling a wider spectrum of lepidopteran pests and help assure the durability of Bt corn relative to the single Cry1Ab protein in MON 810. Control of ECB, ACB, SWCB and SB are comparable for MON 89034 and MON 810 corn. CEW control is improved in MON 89034 corn and FAW control is increased throughout the growing season.

A risk and benefits assessment for re-registration of Bt corn and cotton was prepared by the EPA (U.S. EPA, 2000) and is posted at the following EPA internet site: http://www.epa.gov/scipoly/sap/. 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 Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP). Before lepidopteran-resistant Bt corn varieties were available, some 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. Growers were quick to embrace the use of Bt corn following the commercial availability in 1995. Estimates of Bt corn acreage as a percent of total corn acreage planted are 1% in 1996 to 26% in 1999, 2000, and 2002 (USDA NASS summarized at http://www.usda.gov/nass/pubs/bioc0703.pdf) and 40% in 2006 (USDA NASS summarized at http://www.ers.usda.gov/data/biotechcrops/ExtentofAdoptionTable1.htm).

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 the developers to implement insect resistance management (IRM) strategies. The IRM plan that is currently being used for commercial Bt corn varieties was developed by the National Corn Growers Association in cooperation with biotechnology providers and university entomologists. The plan includes monitoring for compliance with the IRM plan, monitoring for the development of resistant ECB, SWCB, and CEW populations, and mitigation measures in the line that resistant populations are confirmed. The current IRM strategy required for commercially available lepidopteran-resistant Bt corn containing a single Cry protein includes a structured refuge of at least 20% non-Bt corn that may not be sprayed with Bt microbial pesticides and must be planted within ½ mile of the Bt cornfield. In largely cotton growing areas of the south, a refuge of 50% non-Bt corn is required based on considerations of the CEW (aka cotton bollworm). Since the combination of the Cry1A.105 and Cry2Ab2 proteins in MON 89034 corn provides better insect control and a low probability of cross resistance, a reduction in the needed size of the non-Bt refuge may be appropriate. Monsanto has requested a 5% non-Bt refuge in the U.S. Cornbelt and a 20% non-Bt corn refuge in cotton growing regions from the EPA for the FIFRA Section 3 registration of MON 89034. The EPA will require an IRM strategy prior to granting a commercial registration for MON 89034 corn and is considering Monsanto's request for a reduced refuge size than currently required for lepidopteran-active Bt corn (Mike Mendelsohn (EPA/OPP/BPPD) personal communication with Robyn Rose (USDA/APHIS/BRS)). Bt Cry1Ab corn has been in commercial production since 1996 and Cry2Ab has been commercially available in cotton since 2003. There has been no reported lepidopteran insect resistance to the Bt toxins expressed in corn (U.S. EPA, 2000, Tabashnik *et al*. 2003). For corn, this includes ECB, CEW, and SWCB. The lack of documented instances of confirmed insect resistance to Bt corn in the field indicates that the use of mandatory refuges has been an effective tool in preventing or delaying the development of insect resistance to Bt.

Potential impacts of MON 89034 corn on weed control

APHIS evaluated data submitted by the petitioner that show that hybrids derived from MON 89034 corn express Cry1A.105 and Cry2Ab2 proteins. MON 89034 corn is expected to have no impact on current agricultural practices used for weed control as it is no more herbicide tolerant than its non-engineered counterpart.

Volunteers of MON 89034 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 MON 89034 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).

Potential impacts on organic farming and nontransgenic varieties

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.

In 2005, the last year data was available, certified organic corn was grown on 130,000 acres or about 0.16% of the U.S. corn crop (www.ers.usda.gov/Data/organic/Data/certified%20and%20total%20us%20acreage%20selected%20crops%20livestock%2095-05.xls). We have no indication 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* lepidopteran-resistant; and (c) farmers will be educated about recommended management practices and required by the EPA to sign an agreement with Monsanto that they will adhere to IRM requirements and management practices. Moreover, transgenic corn lines resistant to lepidopteran insects are already in widespread use by farmers. Accordingly, this particular product should not present new and different issues than those with respect to impacts on organic farmers in part because so much of the corn crop is already genetically engineered (73% of the 93,000,000 acres planted in 2007) and methods currently exist that allow co-existence of organic corn with a corn crop nearly saturated with GE corn (www.usda.mannlib.cornell.edu/usda/current/Acre/Acre-06-29-2007.pdf). 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 is sufficiently low at 660 feet away to be considered adequate for even the 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 and if appropriately applied should produce the results described above.

F. Cumulative Impacts

As explained above, it is unlikely there will be any direct or indirect significant impacts from the preferred alternative (B). APHIS likewise considered whether the proposed action could lead to any 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. Corn is an annual, wind-pollinated crop which lacks sexually compatible wild relatives (including threatened or endangered plant species) in the U.S. or its territories except for a fairly rare, sparsely dispersed feral population of teosinte that has been reported in Florida. Corn exhibits extremely limited, if any, seed dormancy, has no weedy characteristics, and volunteers are easily controlled. Corn is not observed to be capable of establishing persistent populations in unmanaged environments. The presence of the Bt Cry1A.105 and Cry2Ab2 proteins in MON 89034 corn would not alter cultivation or genetic diversity.

MON 89034 is not the first Bt corn product to be granted nonregulated status. APHIS has previously made determinations of nonregulated status as to other lepidopteran-active Bt corn products and has reached a Finding of No Significant Impact for several EAs prepared for field testing and for deregulation of genetically engineered corn. *See* Determination of Nonregulated Status for MON 801 (60 Fed. Reg. 46107-46108, September 5, 1995), Bt11 (61 Fed. Reg. 2789-2790, January 29, 1996), MON 809 and 810 (61 Fed. Reg. 10720, March 15, 1996), DBT418 (62 Fed. Reg. 17143-17144, April 19, 1997), and Line 1507 (66 Fed. Reg. 42624-42625, August 14, 2001). APHIS evaluated the potential cumulative impacts of granting nonregulated status, in whole, to MON 89034 corn.

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.

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. Based on analysis of data submitted by Monsanto and available in scientific literature, APHIS does not foresee a significant cumulative impact on the ability to maintain adequate genetic purity standards from granting nonregulated status in whole to MON 89034 corn.

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 is 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.

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 currently 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 is 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 disproportionately 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 MON 89034 corn 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 MON 89034 corn described above are expected to have a disproportionately 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", requires Federal agencies to 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. Non-engineered 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 significant 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 non-regulated status be determined for MON 89034 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 non-regulated status under 7 CFR Part 340. Any international traffic in MON 89034 corn subsequent to a determination of non-regulated status for MON 89034 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 Plant Protection Act as well as 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 139 countries are parties to it as of March 5, 2007 (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).

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Appendix A. Additional information on the potential impacts of genetically engineered Bt corn on the human environment.

In general, all plants have the ability to repel, destroy, or mitigate pests. While the mechanisms of plant pest resistance remain a mystery in most cases, all plants are resistant to most pests. In other words, plant pest susceptibility is generally the exception (CAST, 1998). For centuries, farmers and plant breeders have used insect and disease resistance genes from wild relatives to improve crop plants. This is, however, an ongoing process because often insects overcome the resistance.

Agricultural biotechnology has increased the number of ways in which plants can be made resistant to pests. Since the early 1990s, many biotech companies and public institutions (e.g., government and universities) have invested considerable research and development efforts on GE plants resistant to insect pests. To date, only insect-resistant plants expressing genes from *Bacillus thuringiensis* (Bt) have been deregulated by APHIS and registered for commercial use by EPA. Bt proteins have been used for more than 40 years as microbial insecticides, which are sprayed on crop plants. However, their use in commercial agriculture has been limited because the proteins are short-lived in the environment, and sprays can protect only aboveground portions of the plant. Genetic engineering of plants that contain Bt proteins in all tissues continuously throughout the growing season has overcome many of the limitations of Bt microbial insecticides.

Bt is a naturally occurring, Gram-positive bacterium found in many environments including soil, insects, stored-product dust, and deciduous and coniferous leaves. There are two current types of Bt proteins used as insecticides: “crystal” proteins and “vegetative insecticidal” proteins. Crystal proteins, called Cry toxins or delta-endotoxins, form within the spores of Bt bacteria. When ingested by a susceptible insect, these proteins readily bind to receptors on the midgut, insert into its membrane (Gill, Cowles, and Pietrantonio, 1992; Schnepf et al., 1998), and form pores causing destruction of cells, leading to starvation, gut paralysis, septicemia (blood poisoning), and death of the insect (Schnepf et al., 1998).

Commercialization of Bt crops has resulted in fewer insecticide applications and thus, lower management costs (Fitt, 2000; Schnepf et al., 1998; Sankula et al., 2005). Also, one notable advantage of GE insecticidal crops over conventional insecticides is the high specificity of the Bt toxins, which minimizes potential toxic effects on nontarget insects (Betz, Hammond, and Fuchs, 2000; Macintosh et al., 1990). Bt crops may also reduce the need for synthetic insecticides which, in turn, would decrease risks to the environment and effects on nontarget organisms, including beneficial insects.

Assessment of Environmental Effects

Issues that are typically considered in risk assessment of Bt crops include potential effects on non target organisms, potential unintended effects on the target organism, and potential changes in toxicity and allergenicity (Shelton et al., 2002).

Potential Effects on Nontarget Organisms

As the inserted genes code for insecticidal toxins, there is reason to consider in the risk assessment the question of potential effects on nontarget organisms, including beneficial organisms (Pilson and Prendeville, 2004). The scenarios that would be considered are (1) direct effects in the case of other insects or other animals eating the GE plants with the Bt gene, and (2) indirect effects in the case of other animals that consume the target insects due to (a) indirect consumption of the Bt toxin or (b) reduced numbers of prey. In the cases of the GE plants with Bt genes to date, the gene products are well known to specifically target a small group of Lepidoptera or Coleoptera (depending on the specific Bt gene). For example, isopods and earthworms can safely consume Bt corn plant residues (Clark et al., 2006; Vercesi et al., 2006). The likelihood of those insects being directly affected by the Bt toxin depends on the size of crop, that is, in cases of small scale field tests, any impact at the population level of affected insects is very unlikely. In cases of large scale commercial use, the likelihood of nontarget organisms being directly affected depends on whether the nontarget organisms are present and their feeding behavior on the plant in question.

When sensitive insects such as lepidopterans susceptible to Cry1 and Cry2 proteins are not present in the area of planting or do not use the crop involved as their main source of food, then a significant impact at the population level of those insects is very unlikely (Sears et al., 2001). Although one study suggested that pollen from Bt corn event 176 may have adverse impacts on the black swallowtail in the field (Zangerl et al., 2001), there was no evidence in this study or in subsequent studies that large scale effects to swallowtail populations would occur. In addition, expression levels of Bt toxin in the pollen of event 176 are much greater than for MON 89034 or other commercially available corn. Additional studies have verified no significant or adverse affects on non-target insects from Bt corn relative to non-Bt corn particularly when controls involve insecticide sprayed fields (Marvier et al., 2007; Sears et al., 2001; O'Callaghan et al., 2005).

Potential Unintended Effects on the Target Organism

The continuous production of Bt proteins on large acreages may increase the potential for target insects to become resistant to Bt proteins through constant selection pressure on target and nontarget susceptible insects (Tabashnik et al., 2003; Tabashnik et al., 2006). These concerns resulted in the requirement of insect resistance management (IRM) strategies for good stewardship of these crops, and EPA has been the lead Government agency regulating IRM for Bt crops. Written reports on various aspects of IRM are submitted to EPA to aid in the evaluation of the success of resistance management for Bt crops. Although information is often shared between EPA and APHIS, most of the IRM materials and reports are not submitted to or reviewed by APHIS as part of deregulation.

An IRM strategy is developed by incorporating several factors into a single plan to delay resistance of target pests to Bt crops (EPA-SAP, 1995)—

- knowledge of Bt proteins, their targets, and their alternative modes of action,
- knowledge of pest ecology and biology,
- appropriate dosages for Bt proteins,
- appropriate refuge design, and integrated pest management (IPM) of the refuge and Bt crop,
- plans for monitoring, reporting, and mitigating incidents of insect resistance, and
- communication and educational strategies on the use of the product.

Thorough knowledge of pest biology is essential to the effective use of plants expressing Bt proteins and to the management of insect resistance to Bt proteins. For example, feeding behavior of the target pest may influence the optimal location within the plant for Bt protein expression, as well as dosage expression. Larval and adult movement (within and between fields, and in overwintering habitats) may affect the types, sizes, and management of refuges developed for IRM. Reproduction (egg-laying habits, mating preferences, and generations per year) will also influence the development of resistance management plans, particularly when implemented to encourage random mating of insects residing in Bt and non-Bt crops.

Another important component of IRM is determining the effective and appropriate dosage of Bt protein. The February, 1998, FIFRA Scientific Advisory Panel (SAP) Subpanel on *Bacillus thuringiensis* (Bt) Plant-Pesticides and Resistance Management determined that a high-dose strategy, together with a refuge strategy, is necessary to mitigate resistance of stalk-boring Lepidoptera (e.g., moths) in Bt corn (EPA-SAP, 1998). A “high dose” is defined as 25 times the protein dose necessary to kill all susceptible lepidopteran insects (EPA-SAP 1998). For coleopteran (beetle)-active Bt products, the definition of a high dose has not been determined, nor has it been concluded that a high dose is necessary to mitigate resistance for these types of insects.

However, within a population of insects exposed to a high-dose strategy, there may be a few insects resistant to that high dose of Bt protein. If a crop producing that protein is used repeatedly in the same location, it is theoretically possible for these few resistant insects to multiply to form a larger Bt-resistant population. To minimize the incidence of such large Bt-resistant populations, IRM strategies include the use of refuges or refugia.
Structured refuges are areas containing non-Bt host plants. Insects feeding on these plants will not be exposed to the Bt protein, and Bt-resistant insect populations should not develop on these plants. Refuges therefore provide sufficient Bt-susceptible adult insects to mate with any Bt-resistant adult insects that may survive on Bt crop plants. These matings result in Bt-susceptible

offspring which decreases the number of resistant insects and dilutes the frequency of resistance genes (Tabashnik et al., 2004).

Refuge size, proximity to the GE crop, and refuge management are believed critical for resistance management. Refuge size and location must be structured to maximize the potential for mating between susceptible insects (from the refuge) and possible resistant survivors (from the Bt field). Currently, refuges are planted with a similar hybrid, in close proximity to, and concurrently with, the Bt crop (Shelton et al., 2002). Refuges are treated as needed to control insect pests with non-Bt insecticides or other appropriate IPM practices, and managed according to standard practices in the Bt field.

As more Bt products are commercialized, it is theoretically possible for insect pests to come into contact with multiple Bt insecticidal proteins during their development. If the insecticidal proteins produced by the Bt plants all have similar modes of action, pests may develop cross-resistance (resistance to all proteins using that mode of action) (Tabashnik et al., 1994). One potential method to circumvent or delay cross-resistance is to plant two or more Bt crops, each of which produces a Bt protein with a mode of action different from the others. The theory behind spatial refuges is that it is very unlikely that a pest population would develop resistance to multiple unrelated proteins. However, for many pests, a single individual will only experience a single plant and therefore, a single Bt protein (mode of action) during its development. Because many pest larvae do not move from plant to plant and would not be exposed to multiple Bt proteins, spatial refuges have not been implemented. Other methods for decreasing the likelihood of insect resistance is by incorporating two Bt genes with different mechanisms into a single plant, termed stacking (Zhao et al., 2005) or by altering the toxicity of the protein (Mehlo et al., 2005). The chance that an individual in the population would possess resistance to both mechanisms and escape mortality in order to propagate resistance in the population is exponentially lower than with a single Bt gene. This strategy is only effective if both genes are deployed simultaneously, before resistance has developed in the population to overcome either mechanism.

Monitoring and Mitigation

Identifying populations of resistant insects through a comprehensive resistance monitoring plan is one method to test the effectiveness of resistance management programs and detect the onset of resistance before widespread crop failure occurs. However, monitoring and detecting pest resistance to a Bt protein is a difficult and imprecise task requiring a high level of sensitivity and accuracy. Appropriate resistance monitoring requires baseline susceptibility data prior to initiation of a monitoring program. In addition to baseline susceptibility data, information is needed to determine how many individuals need to be sampled and in how many locations. The chances of finding resistant larvae in a Bt crop depend on the level of pest pressure, the frequency of resistant individuals, the location and number of samples that are collected, and the sensitivity of the detection technique.

Because there have been no confirmed instances of pest resistance to Bt crops currently planted, there has been no need to implement mitigation measures, and their success has not been evaluated. Mitigation may involve:

- informing customers and extension agents in the affected areas of suspected or confirmed resistance,
- increasing monitoring in the affected areas,
- implementing alternative means to reduce or control target pest populations in the affected areas,
- implementing a structured refuge in the affected areas, and
- halting Bt seed sales in the affected and bordering counties until an effective local management plan has been implemented.

Grower Stewardship

Growers are an essential element for the implementation and success of an IRM plan as they are responsible for planting refuges according to guidelines, and for monitoring fields for unexpected pest damage. Therefore, an education program that informs growers why IRM is needed and provides guidance on how to implement appropriate strategies is necessary. EPA requires registrants to obtain technology use agreements from growers that outline IRM requirements and acknowledges the growers' responsibility to comply with them. The agreement states that growers received a product use guide provided by the company selling the Bt seed. Technical bulletins, grower guides, sales materials, training sessions, Web sites, toll-free numbers for questions or further information, and educational publications have been recommended as tools to educate growers. Educational materials should be consistent and reflect the most current resistance-management guidelines to help ensure compliance with IRM requirements. It takes time and money to comply with IRM requirements, and there is a concern that if IRM requirements are too complex or time-consuming, growers may avoid planting Bt crops or not adhere to IRM strategies (Langrock et al., 2003).

Potential Changes in Toxicity and Allergenicity to Mammal, Avian, and Aquatic Organisms

EPA-registered Cry proteins have been considered safe because the intestinal walls of mammals do not have the endotoxin receptor necessary for the toxic effect, and the proteins are degraded quickly in the stomach (Sacchi et al., 1986; Shimada et al., 2005; Shimada et al., 2006). Vegetative insecticidal proteins (VIPs) are secreted proteins derived from the vegetative growth stage of Bt. When ingested, the protein binds to midgut cells, attacks the epithelial layer of the midgut, and eventually causes death (Lee et al., 2003). VIPs have a similar mode of action as Cry proteins, but VIPs associate with different midgut binding sites (Cao-Guo et al., 1997; Lee et al., 2003; Yu et al., 1997). Both Bt Cry proteins and VIPs have been deregulated by APHIS, and Cry proteins have been registered for commercial use by EPA.

As part of the ecological risk assessment, EPA also considers potential risks to mammals, birds, and fish. Although wildlife may be exposed to Bt protein, there is no evidence to date that shows toxicity to wild or domesticated mammals, fish, or avian species, and there are no reports of adverse effects from the commercial poultry industry after several years of using Bt corn in poultry feeds (Shimada, 2006a; Taylor et al., 2005). Potential for accidental aquatic exposure from Bt crops is extremely small, and there is no evidence for sensitivity of aquatic species to Bt proteins (EPA–BPPD, 2001). APHIS, as part of its ecological risk assessment, also considers potential risks of GE plants to migratory birds under the Migratory Bird Act and threatened and endangered species under the Threatened and Endangered Species Act.

Appendix B. Summary of habitat and food sources of threatened and endangered Lepidoptera.

Given the specificity of activity of the Cry1A and Cry2A proteins, species outside the insect order Lepidoptera should not be affected. APHIS has thoroughly examined all threatened and endangered lepidopterans and determined that the breeding habitat of these lepidopterans does not overlap corn. Threatened and endangered lepidopterans in the U.S. have very restrictive habitat ranges; and their larvae typically feed on specific host plants, none of which include corn or its sexually compatible relatives. An examination of county distribution of endangered lepidopterans shows that they do not occur in agricultural settings where corn is grown except the Karner blue butterfly (*Lycaeides Melissa samuelis*).

The Karner blue requires wild lupine *(Lupinus perennis)* as an oviposition substrate and larval food source, while the adults feed on wild flowers. As of 1992, Karner blue is known to exist along the northern extent of the range of wild lupine, where there are prolonged periods of winter snowpack, in parts of Wisconsin, Michigan, Minnesota, Indiana, New Hampshire, New York, and Illinois (Haack, 1993). Although there are two counties in Wisconsin that have been identified as having a potential overlap between corn pollen shed and the presence of Karner blue larvae, there is no evidence of Karner blue exposure to corn pollen in these locations. In addition, Monsanto conducted a risk analysis of MON 89034 corn pollen to the Karner blue butterfly that showed a 12-fold margin of safety in the event of the highest possible exposure to corn pollen. It can therefore be concluded that there will be no effect to threatened and endangered lepidopterans including Karner blue butterflies from MON 89034 corn.

a. Lycaenidae

The **El Segundo blue butterfly** (*Euphilotes battoides allyni*) is endangered in four disjunct locations of Los Angeles County, California. They inhabit coastal sand dunes that are suitable for the adult and larval host plant *Eriogonum parvifolium*. They are limited to the El Sgundo sand dunes which is a habitat with high sand content. Known extant populations occur in the Ballona Wetlands, Airport Dunes, Chevron butterfly preserve, and Malaga Cove. A recovery plan has been developed for this species and recommends protection of their habitat, removal of alien plant species and protection from other detriments such as off-road vehicle use. (http://ecos.fws.gov/docs/recovery_plans/1998/980928d.pdf).

The **Fender's blue butterfly** (*Icaricia icarioides fenderi*) is endangered in Oregon where they occur in upland prairies of theWillamette Valley. Larvae predominantly feed on Kincaid's lupine but have also been found on sickle keeled and spurred lupine. This species decline is mostly due to habitat loss from agriculture and urban development. Invasion by alien plant species such (e.g., Himalayan blackberry and Scotch broom) have resulted in degradation of vegetation communities and competition with native lupines. Species recovery requires restoration of degraded prairie habitat to remove non-native plants. (http://www.xerces.org/Pollinator_Red_List/Leps/Icaricia_icarioides_fenderi.pdf#search='Icaricia%20icarioides%20fenderi%2C%20habitat%2C%20natureserve'). There is no recovery plan nor critical habitat identified for this species.

The **Karner blue butterfly** (*Lycaeides melissa samuelis*) formerly occurred in a band extending across 12 states from Minnesota to Maine and in the province of Ontario, Canada, and now only occurs, and is endangered, in the seven states of Minnesota, Wisconsin, Indiana, Michigan, New York, New Hampshire, and Ohio. Wisconsin and Michigan support the greatest number of Karner blue butterflies and butterfly sites. The majority of the populations in the remaining states are small and several are at risk of extinction from habitat degradation or loss. Based on the decline of the Karner blue across its historic range, it was listed as endangered in 1992. Since listing, two populations have been extirpated and are being reintroduced to Concord, New Hampshire, and West Gary, Indiana. A third population is being reintroduced to Ohio. The Karner blue butterfly is dependent on wild lupine, *Lupinus perennis* L. (Fabaceae), its only known larval food plant, and on nectar plants. These plants historically occurred in savanna and barrens habitats typified by dry sandy soils, and now occur in remnants of these habitats, as well as other locations such as roadsides, military bases, and some forest lands. The primary limiting factors are loss of habitat through development, and canopy closure (succession) without a concomitant restoration of habitat. A shifting geographic mosaic that provides a balance between closed and open-canopy habitats is essential for the maintenance of large viable populations of Karner blue butterflies (http://ecos.fws.gov/docs/recovery_plans/2003/030919.pdf).

The **Lange's metalmark butterfly** (*Apodemia mormo langei*) is endangered in California. They occur at the Antioch Dunes in Contra Costa County in association with naked buckwheat. Larvae feed only on naked buckwheat and adults feed on the nectar. Adults also feed on nectar from butterweed and San Joaquin snakeweed and silver lupine is used for mating. Most of this species habitat has been lost and protection of mobile dune systems is needed for protection of naked buckwheat. This species habitat has been depleted by large-scale sand mining and industrial development. Invasion by alien grasses have crowded native plants from the ecosystem and prevented movement of sand which is needed for naked buckwheat to reproduce. Recovery of this species will require habitat improvement through dune restoration, hand-pulling of alien plant species, planting buckwheat seedlings, and restricting public access to areas where they occur.
(http://www.xerces.org/Pollinator_Red_List/Leps/Apodemia_mormo_langei.pdf#search='Apodemia%20mormo%20langei%2C%20habitat'). There is no recovery plan nor critical habitat identified for this species.

The **Lotis blue butterfly** (*Lycaeides argyrognomon lotis*) is endangered in California. Possibly extinct, the Lotis Blue has not been seen alive since 1983. Little is known about this mysterious butterfly. It is only known from a few sites near Mendocino on California's north coast. Thought to have been restricted to a rare coastal bog type of habitat, this butterfly may be a victim of climatic shifts as much as development (http://essig.berkeley.edu/endins/lotis.htm). The lotis blue butterfly probably occurred in wet meadows and sphagnum willow bogs. The suspected food plant for larvae is the coast trefoil, which is relatively common along the Mendocino coast in damp coastal prairie (http://www.fws.gov/arcata/es/inverts/lotisBlue/lotis.html).

The **Mission blue butterfly** (*Icaricia icarioides missionensis*) is endangered in California. The mission blue butterfly was first collected in 1937 from the Mission District of San Francisco. Today a small colony is located on Twin Peaks. The species has also been collected from Fort Baker, Marin County. The majority of the remaining colonies are found on San Bruno

Mountain, San Mateo County. Other colonies have been discovered in San Mateo County. Colonies are located at sites ranging from 690 to 1,180-foot elevation. Some colonies occur in the fog belt of the coastal range. Coastal chaparral and coastal grasslands dominate the vegetation type where colonies are found (http://www.fws.gov/sacramento/es/animal_spp_acct/mission_blue_butterfly.htm).

The **Mitchell's satyr butterfly** (*Neonympha mitchellii mitchellii*) is endangered in Indiana, Michigan and Ohio. Of the more than 30 historical populations known, 15 extant populations are known from Michigan (13) and Indiana (2). Mitchell's satyr habitat is best characterized as a sedge-dominated fen community. Occupied fens are located in a small region of southern Michigan and northern Indiana. Habitat loss and disruption of ecological processes which create and maintain habitat are the probable cause of decline (http://ecos.fws.gov/docs/recovery_plans/1998/980402.pdf).

The **Palos Verdes blue butterfly** (Glaucopsyche lygdamus palosverdesensis) is endangered in California and restricted to the Palos Verdes peninsula, Los Angeles County. The primary threat to this species is overgrowth of weeds and weed control practices adversely affecting the butterfly's food plant, locoweed (*Astrogalus trichopodus leucopsis*) (http://ecos.fws.gov/docs/federal_register/fr433.pdf). This speties is exclusively peninsular, baingrestricted to the cool, fog-shrouded side of Palos Verdes Hills. The only presently known population occupies- several acrea near the intersection of Los Verdes Drive and Hawthorne Boulevard. Accelerated residential and commercial development on the Palos Verdes Peninsula is threatening the continued existence of this species (http://ecos.fws.gov/docs/federal_register/fr224.pdf).

The **San Bruno elfin butterfly** (*Callophrys mossii bayensis*) is endangered in California. This butterfly is limited in occurrence to a few moist-canyons in San Mate County California. Proposed development poses a serious threat to its continued existence. The occurrence of the butterfly is dependent upon present topographic configuration and its caterpillar food Plant, stone-crop (Sedum spathuliffolium) (http://ecos.fws.gov/docs/federal_register/fr76.pdf).

The **Smith's blue butterfly** (*Euphilotes enoptes smithi*) is endangered in California. In addition to their small size, Smith's blue butterflies live for a very short time, for only about one week. Their single week of daytime-only flight is further limited to temperatures above 60 degrees and when there are no strong winds or in areas where they are sheltered from the wind. The overall population of adults is active for about 8 to 12 weeks between early June to September. Another limiting factor is that most butterflies fly less than 200 feet from where their lives began as eggs. Roadways are considered barriers that may isolate Smith's blue butterfly colonies from each other. For thousands of years these tiny butterflies have developed a reliable codependency on just two species of buckwheat that inhabit the coast. They feed, mate, and lay their eggs exclusively on the flower heads of Coast buckwheat (*Eriogonum latifolium*) and Seacliff buckwheat (*Eriogonum parvifolium*). Smith's blue butterfly has resided in the dunes for eons. It is only for the last 50 years that human impacts have caused their endangerment as their buckwheat host plants have begun to get wiped out by human activities (http://www.fws.gov/desfbay/Archives/Smith/Smiths3.htm).

b. Nymphalidae

The **Bay checkerspot butterfly** (*Euphydryas editha bayensis*)is threatened in California and occurs in patches near the San Francisco Bay area. A total of approximately 9,673 hectares (23,903 acres) in San Mateo and Santa Clara counties, California, is designated as critical habitat. Habitat of the bay checkerspot most commonly is found on shallow, serpentine-derived or similarly droughty or infertile soils, which support the butterfly's larval food plants and also includes nectar sources for adults that may also occur on other adjacent soil types. Serpentine soils are high in magnesium and low in calcium, and are a strong indicator of habitat value for the bay checkerspot. The primary larval host plant of the bay checkerspot is *Plantago erecta* (dwarf plantain), an annual, native plantain. The bay checkerspot usually is found associated with *Plantago erecta* in grasslands on serpentine soils, such as soils in the Montara series. In Santa Clara County, the Inks and Climara soil series are related soils and often have inclusions of Montara (U.S. Soil Conservation Service 1974). Henneke and other serpentine soils also occur within the range of the bay checkerspot. The bay checkerspot's life cycle is closely tied to host plant biology. Host plants germinate anytime from early October to late December, and senesce (dry up and die) from early April to mid May. Most of the active parts of the bay checkerspot life cycle also occur during this period. Adults emerge from pupae (a transitional stage between caterpillar and adult butterfly) in early spring, and feed on nectar, mate, and lay eggs during a flight season that typically lasts for 4 to 6 weeks in the period between late February to early May. The eggs hatch and the tiny larvae feed for about 2 to 3 weeks before entering diapause (a temporary cessation of development) in mid to late spring. The postdiapause larvae emerge after winter rains stimulate germination of *Plantago*, and feed and bask until they are large enough to pupate and emerge as adults (Service 1998). If insufficient food is available, a post-diapause checkerspot larva can re-enter diapause and emerge again one year or more later (Singer and Ehrich 1979; Mattoni *et al.* 1997). Most *Euphydryas editha* subspecies exhibit generally sedentary behavior, with adults fequently remaining in the same habitat patch in which they developed as larvae (Ehrlich 1961, 1965; Boughton 1999, 2000). Female bay checkerspots were found to be more likely to emigrate than males (Ehrlich *et al.* 1984). Adult dispersal by the bay checkerspot is typically less than 150 meters (490 feet) between recaptures (Ehrlich 1961, Ehrlich 1965, Gilbert and Singer 1973) . However, Harrison (1989) recaptured bay checkerspots greater than 1 kilometer (0.6 mile) from the point of release in 5 percent of cases and they have been documented at distances as far as 7.6 km (4.7 miles) away. Qualitative observations suggest that bay checkerspots move readily over suitable grassland habitat, but are more reluctant to cross scrub, woodland or other unsuitable habitat (http://ecos.fws.gov/docs/federal_register/fr3740.pdf).

The **Behren's silverspot butterfly** (*Speyeria zerene behrensii*) is endangered in California. The Behren's silverspot butterfly occupies early successional coastal terrace prairie habitat that contains *Viola adunca* (early blue violet), the larval host plant, adult nectar sources, and adult courtship areas. Several populations have apparently been extirpated, and the species likely remains at a single location near Point Arena, Mendocino County, California. It was federally listed as an endangered species on December 5, 1997 (62 FR 64306). Threats include invasion by exotic species, natural succession, fire suppression, residential development, and collection. This draft recovery plan includes conservation measures designed to ensure that a self-sustaining population of Behren's silverspot butterfly will continue to exist, distributed throughout its extant and historic range. Specific recovery actions focus on protection and management of suitable habitat with larval food plants. The draft recovery plan also addresses the need to reestablish multiple populations of Behren's silverspot butterfly within its historic range. The ultimate objective of this recovery plan is to delist Behren's silverspot butterfly through implementation of a variety of recovery actions including: (1) Protecting existing habitat; (2) locating or establishing new metapopulations; (3) developing and implementing management plans; (4) monitoring metapopulations and habitat; and (5) reducing take and sources of mortality (http://ecos.fws.gov/docs/federal_register/fr4214.pdf).

The **Callippe silverspot butterfly** (*Speyeria callippe callippe*) is endangered in California in the San Francisco Bay area. They are found at two sites on grasslands in the San Francisco Bay area and is imperiled by overcollecting, urban development, alien plant invasion and competition, and excessive livestock grazing. The callippe silverspot butterfly is found in native grassland and associated habitats (Thomas Reid Associates 1982; Steiner 1990; Mattoon, *in litt.*, November 22, 1992). The females lay their eggs on the dry remains of the larvae foodplant, Johnny jump-up (*Viola pedunculata*), or on the surrounding debris (Arnold 1981, Thomas Reid Associates 1982). Within about 1 week of hatching the larvae eat their egg shells. The caterpillars wander a short distance and spin a silk pad upon which they pass the summer and winter. The larvae are dark colored with many branching sharp spines on their backs. The caterpillars immediately seek out the foodplant upon termination of their diapause in the spring. In May, after having gone through five instars, each larva forms a pupa within a chamber of leaves drawn together with silk. Adults emerge in about 2 weeks and live for approximately 3 weeks. Depending upon environmental conditions, the flight period of this single-brooded butterfly ranges from mid-May to late July. The adults exhibit hilltopping behavior, a phenomenon in which males and virgin or multiple-mated females seek a topographic summit on which to mate (Shields 1967) (http://ecos.fws.gov/docs/federal_register/fr3183.pdf).

The **Myrtle's silverspot butterfly** (*Speyeria zerene myrtleae*) is endangered in California. Three populations totaling 10,000 individuals were observed from 1988 to 1998. The host plant species are restricted to foredunes and dune scrub vegetation, and to adjacent sandy habitats occupied by coastal scrub or coastal prairie of northern or central California coasts. The butterfly species occurs in the coastal grasslands and scrub, with the host plant (western dog violet) in the immediate vicinity of Point Reyes, Marin County. This species is threatened by competition from non-native plants, loss of habitat from commercial and residential development, and habitat disturbance from recreation and grazing. The Point Reyes dune system is well protected (http://ecos.fws.gov/docs/recovery_plans/1998/980930d.pdf).

The **Oregon silverspot butterfly** (*Speyeria zerene hippolyta*) is threatened in California, Oregon and Washington. The Oregon silverspot butterfly, which was listed as threatened with critical habitat in 1980, is a small, darkly marked coastal subspecies of the Zerene fritillary butterfly. This subspecies occurs in six small pockets of remaining habitat at Del Norte/Lake Earl in California and Clatsop Plains, Mt. Hebo, Cascade Head, Bray Point and Rock Creek-Big Creek in Oregon. A population in Long Beach, Washington may be extirpated and the population on the Clatsop Plains is extremely low and at risk of extirpation. The open vegetation preferred by the butterfly has always had a patchy distribution that was maintained through wildfire, salt-laden winds, grazing, and controlled burning. Habitat has declined due to residential and commercial development, invasion of exotic plant species, overgrazing, and lack of fire. Current threats include continued habitat alteration, continued invasion of non-native plants, off-road vehicle use, and vegetation change due to fire suppression. Recovery of this species requires permanent management of protected habitat in the habitat conservation areas listed in the plan to maintain native, early successional grassland communities which include early blue violet and native nectar species (http://ecos.fws.gov/docs/federal_register/fr3821.pdf).

The **Quino checkerspot butterfly** (*Euphydryas editha quino (=E. e. wrighti)*) is endangered in California and known to occur in the San Diego National Wildlife Refuge. The Quino checkerspot butterfly is found in association with topographically diverse open woody canopy landscapes that contain low to moderate levels of non-native vegetation compared to disturbed habitat. Vegetation types that support the Quino checkerspot are coastal sage scrub, open chaparral, juniper woodland, forblands, and native grassland. Soil and climatic conditions, as well as ecological and physical factors, affect the suitability of habitat within the species' range. Urban and agricultural development, invasion of non-native species, habitat fragmentation and degradation, increased fire frequency, and other human-caused disturbances have resulted in substantial losses of habitat throughout the species' historic range (http://ecos.fws.gov/docs/federal_register/fr4177.pdf).

The **Saint Francis' satyr butterfly** (*Neonympha mitchellii francisci*) is endangered in North Carolina. Saint Francis' satyr is extremely restricted geographically. The northern subspecies has been eliminated from approximately half its known range, primarily due to collecting (Refsnider 1991). Saint Francis' satyr is now known to exist as a single population in North Carolina. The annual life cycle of N. m. francisci, unlike that of its northern relative, is bivoltine. That is, it has two adult flights or generations per year. Larval host plants are believed to be graminoids such as grasses, sedges, and rushes. Little else is known about the life history of this butterfly. The habitat occupied by this satyr consists primarily of wide, wet meadows dominated by sedges and other wetland graminoids. In the North Carolina sandhills, such meadows are often relicts of beaver activity (http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=1995_register&docid=fr26ja95-13).

The **Uncompahgre fritillary butterfly** *(Boloria acrocnema)* is endangered in Colorado. In 1990, this butterfly was described to have the smallest total range of any North American butterfly species and is only found in remote, generally inaccessible areas. Its habitat is limited to two verified major sites and two possible small colonies in the San Juan Mountains in Hinsdale County in southwestern Colorado. One major site is the type locality on Mount Uncompahgre, which is managed by the Forest Service. The second major site was discovered in 1982 on land managed by the Bureau of Land Management and is not generally known. All known populations are associated with large patches of snow willow above 4,040 m (13,200 ft) which provide food and cover (http://ecos.fws.gov/docs/federal_register/fr1776.pdf).

c. Papilionidae

The **Schaus swallowtail butterfly** (*Heraclides aristodemus ponceanus*) is endangered in Florida and known to occur in the Crocodile Lake National Wildlife Refuge. The Schaus swallowtail originally occurred from the Miami area south through the Florida Keys as far as Lower

Matecumbe Key. The last records from Miami were in 19%. Presumably, urban development eliminated the habitat of the species there. The last records for Upper and Lower Matecumbe Keys were in the mid-‘1949’s. The disappearance of the species from these Keys apparently coincided with heavy collecting pressure, although collecting is not known to have caused the decline. In the early 1979’s, the butterfly was relatively abundant on north Key Largo, but appears to be rare there now. The known range of the Schaus swallowtail is now Elliott and Old Rhodes Keys in Biscayne National Park, Dade County, and north Key LArgo, Monroe County (Loftus and Kushlan, 1982; U.S. Fish and Wildiife Service, 1982). The Schaus swallowtail is restricted to tropical hardwood hammocks, which constitute the climax vegetation of upland areas in the Florida Keys. Formerly, this vegetation type occurred more widely in south Florida, but has been largely eliminated on the mainland. The hammocks are closely related floristically to the West Indies, and constitute the only tropical upland continental U.S. The Florida Keys contain the largest remaining hammocks, but many-of the areas are highly subject to development pressures because of restrictions on development in the surrounding lowland (mangrove) areas. Local, State, and Federal laws presently limit development on these wetlands. The hammocks contain a large number of plant species rare to Florida, many of which are considered threatened or endangered by this State. The tropical hardwood hammock plant community is considered to be one of the most restricted and vulnerable habitat types in the U.S. (http://ecos.fws.gov/docs/federal_register/fr881.pdf).

d. Sphingidae

The **Blackburn's sphinx moth** (*Manduca blackburni*) is endangered in Hawaii. Larvae of Blackburn’s sphinx moth feed on plants in the nightshade family (Solanaceae). The natural host plants are native shrubs in the genus *Solanum* (popolo), and the native tree, *Nothocestrum latifolium* (‘aiea) (Riotte 1986), on which the larvae consume leaves, stems, flowers, and buds (B. Gagne´, pers. comm. 1994). However, many of the host plants recorded for this species are not native to the Hawaiian Islands, and include *Nicotiana tabacum* (commercial tobacco), *Nicotiana glauca* (tree tobacco), *Solanum melongena* (eggplant), *Lycopersicon esculentum* (tomato), and possibly *Datura stramonium* (Jimson weed) (Riotte 1986). Development from egg to adult can take as little as 56 days Williams 1947), but pupae may remain in a state of torpor (inactivity) in the soil up to a year (Williams 1931; B. Gagne´, pers. comm. 1994). Adult moths can be found throughout the year (Riotte 1986) (http://ecos.fws.gov/speciesProfile/SpeciesReport.do?spcode=I0AL). Historical records of this species are mostly from coastal, lowland, and dryland forests in areas receiving less than 50 inches (120 centimeters) of rainfall, though they have been collected from sea level to 2,500 feet (760 meters). It was most common historically on Maui. Many of the host plants recorded for the species are not native to the Hawaiian Islands, and include tree tobacco, commercial tobacco, and tomato plants. Once found on six Hawaiian islands, the moth now exists only on Maui, Kaho`olawe, and the island of Hawai`i. They were believed extinct until 1984 when a small population was rediscovered in a lowland dry forest on the south coast of East Maui (Kanaio area). Additional small isolated populations are now known from other parts of Maui. Populations were recently discovered on Kaho`olawe (the first record of this species on this island) in 1997 and in 1998 in North Kona on the island of Hawai`i. Threats to Blackburn’s Sphinx Moth include introduced ants and parasitic wasps that prey on the eggs and caterpillars, and the loss of its native host plant, `aiea, which is a dryland forest tree. The native host plant is found in endangered ecosystems, dry and mesic forests, and has been adversely affected by feral animals, alien plant invasions, and habitat conversions associated with development (http://www.fws.gov/pacificislands/wesa/sphinxmoth.html).

The **Kern primrose sphinx moth** (*Euproserpinus euterpe*) is threatened in California. This moth is known to occur only in one small area of approximately 6.1 ha (5 acres) in the Walker Bain of Kern County, California, and was thought to be extinct prior to its rediscovery in 1974. The Walker Basin is at an elelvation of 1,470 m in the southern Sierra Nevada. The basin is surrounded by mountains over 2000 m in elevation. A large portion of the basin is devoted to agriculture (prmariliy barley cultivation and cattle pasture). The dominant vegetation in the sandy washes in which the colony occurs includes filaree, bab blue-eyes, and rabbit brush (*Chrysothamnus nauseosus*), as well as gold fields (*Lasthenia chrysostoma*) and Brome grass (*Bromus arenarius*). The soil originates from decomposed granite ans is largely alluvial in nature. Its texture is coarse to fine sand with very little silt. The annual evening primrose, on which the larvae of Kern primrose sphinx moths feed, occurs in dry, disturbed and sandy-gravelly areas below 3000 m in many plant communities from Oregon to Baja California. The flight season of the Kern primrose sphinx moth extends from late February to early April with the peak period during mid-March (http://ecos.fws.gov/docs/recovery_plans/1984/840208.pdf).

e. Hesperidae

The **Carson wandering skipper** (*Pseudocopaeodes eunus obscurus*) is endangered in California and Nevada. The Carson wandering skipper is currently known from only two populations, one in Washoe County, Nevada, and one in Lassen County, California. Carson wandering skipper habitat is characterized as lowland grassland habitats on alkaline substrates. Occupied areas are located in a small region east of the Sierra Nevada in northwestern Nevada and northeastern California, and are characterized by an elevation of less than 1,524 meters (5,000 feet), the presence of *Distichlis spicata* (saltgrass) (Hickman 1993) and nectar sources in open areas near springs or water, and possible association with geothermal activity. Threats to the subspecies include habitat destruction, degradation, and fragmentation due to urban and residential development, wetland habitat modification, agricultural practices, gas and geothermal development, and nonnative plant invasion. Other threats include collecting, excessive livestock trampling/grazing, water exportation projects, road construction, recreation, pesticide drift, and inadequate regulatory mechanisms. This subspecies is also vulnerable to chance environmental or demographic events, to which small populations are particularly vulnerable. The combination of only three populations, small range, and restricted habitat makes the subspecies highly susceptible to extinction or extirpation from a significant portion of its range due to random events such as fire, drought, disease, or other occurrences (Shaffer 1981, 1987; Meffe and Carroll 1994) (http://ecos.fws.gov/docs/recovery_plans/2006/060302.pdf).

The **Laguna Mountains skipper** (*Pyrgus ruralis lagunae*) is endangered in California. The Laguna Mountains skipper is found in montane meadow habitats. The Laguna Mountains skipper is restricted to the Laguna Mountains and Mount Palomar in San Diego County. *Horkelia clevelandii* is the larval host plant of the Laguna Mountains skipper. This plant occurs in meadows, under pines, and on granite in the Laguna, Cuyamaca, Palomar, and San Jacinto Mountains of southwestern California and northwestern Baja California, Mexico, from 1,200 to 2,500 meters (m) (4,000 to 8,000 feet (ft)) in elevation (Hickman 1993). Although the distribution of a butterfly is primarily defined by the presence of its larval host plant, the butterfly may be further restricted by other physiological or ecological constraints. The Laguna Mountains skipper is currently found in a few open meadows of yellow pine forest between 1,200 and 2,000 m (4,000 and 6,000 ft) in elevation (http://ecos.fws.gov/docs/federal_register/fr3034.pdf).

The **Pawnee Mountain skipper** (*Hesperia leonardus montana*) is threatened in Colorado and only occurs on the Pikes Peak Granite Formation in the South Platte River drainage system involving portions of Jefferson, Douglas, Teller, and Park Counties. This skipper occurs in dry, open, Ponderosa pine (*Pinus ponderosa*) woodlands at an elevation range of 6,000 to 7,500 ft. The slopes are moderately steep with soils derived from Pikes Peak granite. The understory is limited to the pine woodlands. Blue gamma grass (*Bouteloua gracilis*), the larval food plant, and the prairie gayfeather (*Liatris punctata*), the primary nectar plant, are two necessary components of the ground cover strata. The vegetative community preferred by the skipper is a northern-most extension of the ponderosa pine/blue gamma grass habitat type documented from Southern Colorado and northern New Mexico (http://ecos.fws.gov/docs/recovery_plans/1998/980921.pdf).

Appendix C. Glossary

Abiotic Stressor: A nonliving factor that causes stress (e.g. to plants) such as cold, drought, flooding, salinity, ozone, toxic metals, and ultraviolet-B light.

Adsorbed: In relation to chemicals, the solute is retained to the surface of an adsorbent. (e.g. chemicals "adhering to soil particles). (Merriam-Webster)

Agronomic: A branch of agriculture dealing with field-crop production and soil management. (Merriam-Webster)

Bacillus thuringiensis (Bt): Discovered by bacteriologist Ishiwata Shigetane on a diseased silkworm in 1901. Later discovered on a dead Mediterranean flour moth, and first named *Bacillus thuringiensis,* by Ernst Berliner in 1915. Today, Bt. refers to a group of rod shaped soil bacteria found all over the earth, that produce "cry" proteins which are indigestible by – yet still "bind" to – specific insects' gut (stomach) lining (epithelium cell receptors), so those "cry" proteins are thereby toxic to certain classes of insects (corn borers, corn rootworms, mosquitoes, black flies, some types of beetles, etc.), but are harmless to all mammals. At least 20,000 strains of Bt. are known. (Nill)

Biopesticides: A compound that kills organisms by virtue of specific biological effects rather than as a broader chemical poison. Differ from biocontrol agents in being passive agents, whereas biocontrol agents actively seek the pest. The rationale behind replacing conventional pesticides with biopesticides is that the latter are more likely to be selective and biodegradable. (FAO)

Chlorpyrifos: Chlorpyrifos is a broad-spectrum organophosphate insecticide. While originally used primarily to kill mosquitoes, it is no longer registered for this use. Chlorpyrifos is effective in controlling cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, and lice. It is used as an insecticide on grain, cotton, field, fruit, nut and vegetable crops, as well as on lawns and ornamental plants. It is also registered for direct use on sheep and turkeys, for horse site treatment, dog kennels, domestic dwellings, farm buildings, storage bins, and commercial establishments. Chlorpyrifos acts on pests primarily as a contact poison, with some action as a stomach poison. It is available as granules, wettable powder, dustable powder and emulsifiable concentrate. (http://extoxnet.orst.edu/pips/chlorpyr.htm)

Coleopteran: Any member of the insect order Coleoptera, consisting of the beetles and weevils. It is the largest order of insects, representing about 40 percent of the known insect species. (http://www.britannica.com/eb/article-9105966/coleopteran)

Diploid: The state of a cell in which each of the chromosomes except for the sex chromosomes, is always represented twice (46 chromosomes in humans). In contrast to the haploid state in which each chromosome is represented only once. (Nill)

Donor Organism**:** The organism from which genetic material is obtained for transfer to the recipient organism.

Fluazifop/Fomesafen: Fluazifop-p-butyl is a selective postemergence phenoxy herbicide used for control of most annual and perennial grass weeds in cotton, soybeans, stone fruits, asparagus, coffee, and others. It may often be used with an oil adjuvant or nonionic surfactant to increase efficiency. It has essentially no activity on broadleaf species. It is compatible with a wide variety of other herbicides and may also be found in formulations with other products such as fenoxaprop ethyl ester (in Horizon and Fusion) and fomesafen (in Tornado). It is available as an emulsifiable concentrate. (http://extoxnet.orst.edu/pips/fluazifo.htm)

Foliarly: Relating to the leaf or leaves.

Introgression: The introduction of new alleles or gene(s) into a population from an exotic source, usually another species. This is achieved by repeated backcrossing of the initial hybrid in order to eliminate all genetic changes except for the desired new gene(s). (Nill)

Glycosylated: This process has taken place when a chemical undergoes a reaction in which glycosyl groups are added to a protein to produce a glycoprotein. (Merriam-Webster)

Glycosylation Analysis: A process of verifying certain proteins are present in order to confirm gene insertion was successful or not. Can include methods such as High Performance Liquid Chromatography and Mass Spectrometry. (www.diss.fu-berlin.de/2006/375/Chapter_1.pdf)

Hemipteran: Plural of order Hemiptera; insect characterized by sucking mouthparts consisting of hinged stylets (mandibles and maxillae) resting in a dorsally grooved rostrate labium. Some common names of insects in this order are stink bugs, assassin beetle, giant water beetle, bug nymphs, box-elder bug, and red bug. (Torre-Bueno, insects.org)

Lepidopteran: Order within the Holometabola (Insecta), including moths and butterflies, characterized by adults with 2 pairs of membranous wings clothed on both surfaces with usually overlapping scales and possessing eruciform larvae. (Torre-Bueno)

Lepidopteran Pests: In this document, it refers to corn pests of the order lepidopteran, such as the Asian corn borer (ACB; *Ostrinia furnacalis*), southwestern corn borer (SWCB; *Diatraea grandiosella*), sugarcane borer (SCB; *Diatraea saccharalis*), fall armyworm (FAW; *Spodoptera frugiperda*) and corn earworm (CEW; *Helicoverpa zea*).

Lyophilized: The removal of water as vapour from frozen material under vacuum. Used to measure water content and to preserve samples, particularly spores. Unlike oven-drying, bound water remains associated with the specimen; syn: Freeze-dry. (FAO)

MALDI-TOF MS**:** Acronym for *Matrix-Associated Laser Desorption Ionization Time of Flight Mass Spectrometry*. A mass spectrometry methodology/technology that can establish, in seconds, the identity, purity, etc. of a sample of proteins, oligonucleotide, or (poly)peptides. This technique is also the identification of gram-positive microorganisms, or used for characterization of genetic materials (DNA, RNA, etc.) on hybridization surfaces. MALDI-TOF utilizes measurement of the time for particles (e.g. proteins) to transit a specific distance after being "dislodged" from ('adhered') surface by a specific amount of energy to precisely determine the molecular weight (of proteins, etc.). (Nill)

Non-target organism**:** An organism which is affected by a treatment (e.g. pesticide application) for which it was not the intended recipient. (FAO)

N-terminal sequence analysis: A method to determine the first few amino acid residues of a protein.

Organophosphate insecticide: One of the most widely used insecticide group, functions by inhibiting Cholinesterase, which is required for the proper function of animals' nervous systems. (extoxnet)

Oviposition Substrate: Material in which an insect lays/deposits eggs on.

Permethrin: A broad spectrum synthetic pyrethroid insecticide, used against a variety of pests, on nut, fruit, vegetable, cotton, ornamental, mushroom, potato, and cereal crops. It is used in greenhouses, home gardens, and for termite control. It also controls animal ectoparasites, biting flies, and cockroaches. It may cause a mite buildup by reducing mite predator populations. Permethrin is available in dusts, emulsifiable concentrates, smokes, ULV (ultra-low volume), and wettable powder formulations. (http://extoxnet.orst.edu/pips/permethr.htm)

Plant-incorporated Protectant (PIP): Pesticidal substances produced by plants and the genetic material necessary for the plant to produce the substance. Genetic material that can make a plant produce certain pesticidal substances is naturally found in and transferred between different plants, but it has also been found in and successfully transferred between entirely different types of organisms using techniques of modern biotechnology. For example, Bacillus thuringiensis or "Bt" is a naturally occurring bacterium that creates a protein which repels or kills certain insects. Scientists can take the gene that produces the Bt pesticidal protein and introduce it into the genetic material of a plant. Once introduced, the plant also begins to make the pesticidal protein, controlling pests when they try to feed on the plant. (http://www.epa.gov/pesticides/biopesticides/pips/index.htm)

Proteolytic: Having the ability to degrade protein molecules. (FAO)

Pyrethroids: Group of synthetic pesticides similar to the natural pyrethrum which is produced from chrysanthemum flowers. (http://extoxnet.orst.edu/pips/pyrethri.htm)

Recipient Organism: The organism which receives genetic material from a donor organism.

SDS-PAGE: Abbreviation for *Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis*. (FAO)

Teosinte: A wild plant (*Zea diploperennis*), native to the country of Mexico, which is related to (domesticated) corn/maize (*Zea mays* L.). (Nill)

Tetraploid: Organism or tissue whose cells contain four haploid sets of chromosomes. (FAO)

Vector or Vector Agent: Organisms or objects used to transfer genetic material from the donor organism to the recipient organism.

Western Blot Analysis: A test performed on biological samples to detect a particular protein not specific for AIDS. Gel electrophoresis is used to separate the proteins in the sample. Next the protein bands (resulting from the gel electrophoresis) are exposed to an antibody that will stick to specific individual protein (bands) which are then identified (as being present in the sample) via dyes. (Nill)