USDA/APHIS Decision on Monsanto Petition 04-125-01P Seeking a Determination of Nonregulated Status for Bt *cry3Bb1* Insect Resistant Corn Line MON 88017

Environmental Assessment

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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 04-125-01p) from the Monsanto Company (hereafter referred to as Monsanto) regarding the regulatory status of genetically engineered (transformed) coleopteran insect-resistant corn derived from their transformation event MON 88017 (designated hereafter as MON 88017 corn). 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 88017 corn have been conducted under permits issued or notifications acknowledged by APHIS. Monsanto petitioned APHIS requesting a determination that MON 88017 corn does not present a plant pest risk, and therefore MON 88017 corn and its progeny derived from crosses with other nonregulated corn should no longer be regulated articles under these APHIS regulations.

The MON 88017 corn has been genetically modified to express a modified *Cry3Bb1* gene from *Bacillus thuringiensis (Bt)* subsp. *kumamotoensis.* This gene encodes an insecticidal protein that protects the corn plants against the feeding damage of larvae of the corn rootworm complex comprised of *Diabrotica* species. As with the previous Bt Cry proteins, Cry3Bb has a high degree of specificity for the target pest. MON 88017 corn also expresses in plants the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. Strain CP4 (CP4 EPSPS), which confers tolerance to glyphosate, the active ingredient in Roundup agricultural herbicides. A fragment containing the *Cry3Bb1* and *cp4 epsps* gene expression cassettes were excised from the plasmid vector and introduced into the corn genome using *Agrobacterium*-mediated transformation.

Field trials of MON 88017 corn have been conducted under the APHIS notification procedure (7 CFR Part 340.3). Performance standards for such field trials require that the regulated article and its offspring must not persist in the environment after completion of the test. In accordance with APHIS procedures for implementing the National Environmental Policy Act (NEPA) (7 CFR Part 372), this EA has been prepared prior to issuing a determination of nonregulated status for MON 88017 corn in order to specifically address the potential for impact to the human environment through the unconfined cultivation and use in agriculture of the regulated article.

II. BACKGROUND

A. Development of MON 88017 corn.

Monsanto has submitted a "Petition for Determination of Non-regulated Status" to the USDA, APHIS (APHIS number 04-125-01p) for genetically engineered corn plants that are resistant to the feeding damage caused by a coleopteran pest complex known as corn rootworms (CRW). A complex of *Diabrotica* species comprise what is known as the CRW complex. The CRW complex includes: western corn rootworm (WCRW: *D. virgifera virgifera*), Mexican corn rootworm (MCRW: *D. virgifera zeae*), northern corn rootworm (NRCW: *D. barberi*) and the southern corn rootworm (SCRW*:* *D. undecimpunctata howardi*). The larvae damage corn by feeding on the roots, thereby inhibiting the ability of the plant to absorb water and nutrients from the soil (Reidell, 1990). This leads to harvesting difficulties due to lodging of the weakened plants (Spike and Tollefson, 1991). CRW is one of the most destructive pests to corn and accounts for a significant quantity of synthetic pesticide usage, with over 15 million acres being treated with organophophate, carbamate, and pyrethroid insecticides in the year 2000. Seasonal losses due to CRW have been estimated at a billion dollars when taking into account both the costs of chemical controls and crop losses. Monsanto has submitted data indicating economically significant levels of control against the two most destructive pests of the complex, WCRW and NCRW, and a somewhat lower but still statistically significant level of control for the less important SCRW.

Monsanto used recombinant DNA techniques to produce and introduce into corn a restriction fragment containing the *cry3Bb1* gene from *Bacillus thuringiensis* subsp. *kumamotoensis*, thereby rendering the corn line resistant to CRW. Regulatory elements for the *cry3Bb1* gene were derived from the plant pathogenic cauliflower mosaic virus (CaMV), and from rice and wheat. The coding and regulatory sequences for the plant selectable marker *cp4 epsps* from *Agrobacterium*, which confers resistance to the herbicide glyphosate, was also introduced on the fragment. Regulatory sequences associated with the c*ry3Bb1* gene or the *cp4 epsps* marker gene are not transcribed and do not encode proteins. The DNA was introduced into corn cells using disarmed, binary *Agrobacterium tumefaciens* transformation with plasmid vector PV-ZMIR39. Plant cells containing the introduced DNA were then selected by culturing on medium containing carbenicillin to eliminate *Agrobacterium*, and glyphosate to eliminate those cells that were not transformed, so that only cells containing the T-DNA survived. Because the transformed cells contain some sequences from plant pathogens, they are explicitly subject to regulation under 7 CFR Part 340.

MON 88017 corn has been field tested in the United States since 1999 as authorized by USDA notifications listed in Appendix G. The list is comprised of more than 200 sites in diverse regions of the U.S. including the major corn growing areas of the Midwest and winter nurseries in Hawaii and Puerto Rico. Field tests conducted under APHIS oversight allow for evaluation in a natural agricultural setting while imposing measures to minimize the risk of persistence in the environment after the completion of the test. Data are gathered on multiple parameters and are used by the applicants to evaluate agronomic characteristics and product performance and are used by APHIS to determine if the new variety poses a plant pest risk.

B. APHIS Regulatory Authority.

APHIS regulations under 7 CFR Part 340, which are promulgated pursuant to authority granted by the Plant Protection Act (Title IV, Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. 7701-7772) regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. 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. MON 88017 corn has been considered a regulated article because some noncoding DNA regulatory sequences were derived from plant pathogens.

Section 340.6 of the regulations, entitled "Petition for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it is derived, the Agency can grant the petition in whole or in part. Therefore, APHIS permits or notifications would no longer be required for field testing, importation, or interstate movement of that article or its progeny.

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

MON 88017 corn is also subject to regulation by other agencies. The EPA is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including herbicides, be registered before distribution or sale, unless exempt by EPA regulation. On March 19, 2001, the EPA announced the receipt of an application filed by Monsanto to register the pesticide product *Bt* Cry3Bb protein and the genetic material necessary for its production in corn (66 *FR* 15435-15436, March 19, 2001). This active ingredient is not included in any previously registered product. 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 March 31, 2004, EPA granted a tolerance exemption for Cry3Bb1 (69 *FR* 16809-16814, March 31, 2004). The exemption concluded that there was a reasonable certainty of no harm from consumption of the protein, as it is digestible in gastric fluid and not considered an allergen.

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 MON 88017 assessment indicating no changes in composition, safety or other relative parameters to FDA on March 30, 2004, and submitted additional information on May 18, June 22 and October 18, 2004. The consultation for MON 88017 corn as food and feed was completed on January 12, 2005 indicating that the FDA has no unresolved issues with respect to marketing of MON 88017 for human food and animal feed.

III. PURPOSE AND NEED

In compliance with the National Environmental Policy Act (NEPA) of 1969 (42 U.S.C. 4321 *et seq*.) and the pursuant implementing regulations (40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372), APHIS has prepared this EA before making a determination on the status of MON 88017 corn as a regulated article under APHIS regulations. The developer of MON 88017 corn, Monsanto, submitted a petition requesting that APHIS make a determination that corn transformation event MON 88017, and any progeny derived from crosses of event MON 88017 with other nonregulated corn varieties, no longer be considered regulated articles under 7 CFR Part 340.

IV. ALTERNATIVES

A. No Action: Continuation as a Regulated Article

Under the "no action" alternative, APHIS would come to a determination that MON 88017 corn and its progeny should continue to be regulated under 7 CFR Part 340. Permits or acknowledgment of notifications from APHIS would still be required for their introduction. APHIS would choose this alternative if there were insufficient evidence to demonstrate lack of plant pest risk from the uncontained cultivation of MON 88017 corn and its progeny.

B. Determination of Nonregulated Status

Under this alternative, MON 88017 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 88017 corn or its progeny. A basis for this determination would be established, which would result in a Finding of No Significant Impact (FONSI) under NEPA. Unrestricted cultivation of the lines would be permitted by APHIS. Such a determination, however, does not preclude any restriction on the cultivation of this corn that might be placed by other regulatory agencies also having authority.

C. Determination of Nonregulated Status, in Part

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

Approval of some but not all of lines requested in the petition. In some petitions, applicants request de-regulation of lines derived from more that one independent transformation event. In these cases, supporting data must be supplied for each line. APHIS could approve certain lines requested in the petition, but not others.

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

V. POTENTIAL ENVIRONMENTAL IMPACTS

The potential environmental impacts of alternatives A and B, as described above in section IV are presented in this section.

Alternative A, Non Action.
In a decision to choose alternative A., no action, MON 88017 (OECD unique identifier MON-88017-3) corn plants would still require APHIS authorization to be planted. In this case measures would need to continue to be implemented to ensure physical and reproductive confinement of MON 88017 corn and any progeny derived from it.

If growers do not have improved varieties of corn seed derived from corn line MON 88017, they may choose to plant another cultivar with similar properties as an alternative, or they may use other chemical or biological control mechanisms or management practices if they feel that their coleopteran pest pressure and weed pressure are high enough to warrant it.

Another deregulated transgenic coleopteran resistant corn expressing Bt Cry3Bb1 delta-endotoxin (YieldGard® Rootworm) was deregulated in 2002. YieldGard® Rootworm was planted on about 0.5% of total corn acreage in 2003, limited by seed supply (Sankula and Blumenthal 2004). Adoption of this CRW resistant corn line is expected to continue to increase in the coming years as more seed becomes available to growers (Sankula and Blumenthal 2004). Other herbicide tolerant corn varieties are also available from seed companies, and have been widely adopted by farmers in the United States (Sankula and Blumenthal 2004; Fernandes-Cornejo and McBride, 2000; Carpenter and Gianessi, 1999). Herbicide tolerant varieties include the transgenic Liberty Link® varieties resistant to the herbicidal active ingredient glufosinate-ammonium (e.g. as found in the herbicide Liberty ® which is registered in the United States for use on seed designated as Liberty Link®), transgenic Roundup Ready® varieties resistant to the herbicidal active ingredient glyphosate (as found in the herbicide Roundup®), as well as non-transgenic varieties resistant to two other types of herbicides: the acetolactate synthase (ALS) inhibiting herbicide imidazolinone (IMI) and sethoxydim (Knake, 1998; Fernandez-Cornejo and McBride, 2000). Several chemical insecticides and biological or cultural control measures can be used to control the insect pests targeted by MON 88017 corn, and several herbicides and cultural practices can be used to manage weeds in corn. However, there are no corn products currently available that combine both CRW resistance and glyphosate or glufosinate-ammonium tolerance.

Corn rootworms are the most serious insect pests in field corn in the U.S., costing growers millions of dollars each year in terms of insecticide use and crop loss (Sankula and Blumenthal, 2004). Historically, crop rotation has provided effective protection from CRW damage. More recently, however, the effectiveness of crop rotation has become more limited because of several factors:

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

al., 2002).

2. Crop rotation is not an effective management strategy for southern corn rootworm (SCRW) because it not only has a wide host range, but also because multiple generations can be produced in the same cornfield (Gianessi *et al.* 2002). Larvae of SCRW can be found on the roots of corn, peanuts, alfalfa and cucurbits. There may be two to three generations of SCRW per year. Adults become active and lay eggs in the soil in late spring. These eggs hatch after one week and the larvae feed on corn roots for two to four weeks before pupating. A new generation of adults can emerge in mid-summer (Gianessi *et al.* 2002).

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

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

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

If growers do not have improved varieties of corn seed derived from event MON 88017, they may choose to plant another cultivar with similar properties as an alternative, or they may use other chemical or biological control mechanisms or management practices. APHIS envisions no significant adverse impacts over and above those associated with current practices.

Alternative B, Determination of Nonregulated Status.
A decision to choose alternative B, deregulation of MON 88017 corn, is addressed below. The unrestricted cultivation and distribution of MON 88017 corn is compared to that for other corn not subject to regulation by APHIS under 7 CFR Part 340.

A. Plant Pathogenic Properties

APHIS considered the potential for the transformation process, the introduced DNA sequences, or their expression products to cause or aggravate disease symptoms in MON 88017 corn or other plants or to cause the production of plant pathogens. We also considered whether data indicate that unanticipated plant pest effects would arise from cultivation of MON 88017 corn.

For the transformation process, A x Hi-II plant cells were transformed using *Agrobacterium*-mediated transformation. The publicly-available inbred Hi-II germplasm was obtained from the Maize Genetics Stock Center. This inbred line was derived by crossing the publicly available B73 and A188 Stiff Stalk inbreds, and is intended for use in corn transformation. Inbred A is a private elite Stiff Stalk inbred that is commonly used as a female in breeding with other corn inbreds. Immature embryos of A x Hi-II corn tissue were transformed using *Agrobacterium tumefaciens* strain AbI containing the plasmid PV-ZMIR39. Strain ABI contains a disarmed Ti plasmid that is incapable of inducing tumor formation. Plasmid PV-ZMIR39 (Figure III-1, page 40 of petition) contains the left and right transfer DNA (T-DNA) border sequences that facilitate transformation.

APHIS analyzed data that demonstrates that MON 88017 corn plants regenerated from the transformation event designated MON 88017 contains one, intact copy of the following genetic elements from the plasmid PV-ZMIR39: the left and right border sequences; (left boarder to right border) *cp4 epsps* coding sequence from *Agrobacterium tumefaciens*, joined to an *Arabidopsis thaliana* chloroplast transit peptide (CTP2) sequence, regulated by the 5' noncoding end of the rice actin sequence (ract1) containing the promoter and first intron, and the nopaline synthase 3' polyandenlyation sequence (NOS 3'); the cry 3Bb1 coding region regulated by the enhanced 35S promoter (e35s), a 5' untranslated leader of wheat chlorophyll a/b/-binding protein (wt CAB leader), the 5' untranslated leader of wheat chlorophyll a/b/-binding protein (wt CAB leader), the ract1 intron, and the 3' nontranslated region of the coding sequence for wheat heat shock protein 17.3 (tahsp17 3').

Table IV-1 (pages 45-47 of Petition) lists each of the genetic elements found in PV-ZMIR39. Figures IV-1 and IV-2 (pages 48-49 of Petition) show the deduced amino acid sequences of the CP4 EPSPS and Cry3Bb1 proteins, respectively. The deduced CP4 EPSPS sequence (Figure IV-1) includes the CTP2 transit peptide sequence. The deduced Cry3Bb1 amino acid sequence designates the six amino acids that are different from wildtype Cry 3Bb1. Figures V-1a V-1b (pages 51-52 of petition) show maps of the genetic elements in PV-ZMIR39, as well as restriction enzyme sites and probes used to confirm the genetic analysis. Figure V-2 (page 53 of petition) provides a schematic linear map of the genetic elements contained in the insert present in MON 88017, as well as restriction enzyme sites used for Southern blot analysis, and sizes of predicted restriction fragments. Confirmation of a singe copy of the insert in MON 88017 is provided in Figure V-3 (page 55 of Petition). Southern blot analysis data shown in Figures V-4 through V-10 (pages 57-67 of Petition) provides evidence for intactness of each of the genetic elements in PV-ZMIR39. Finally, the Southern blot analysis shown in Figure V-11 (page 68 of Petition) confirms the lack of plasmid backbone or other sequences from the transformation vector PV-ZMIR39 in the MON 88017 transformed plants.

Although CaMV and *A. tumefaciens* are plant pathogens, the sequences included in MON 88017 corn cannot cause plant disease. They do not encode infectious entities and serve a purely regulatory function for the genes of interest. In addition, these sequences have a history of safe use in genetically engineered plants. The donor organism for the *cry3Bb1, Bacillus thuringiensis* subsp. *kumamotoensis,* is a soil-inhabiting bacterium and is not a plant pathogen. The wildtype *cry3Bb1* coding sequence was modified to encode six specific amino acid substitutions from wildtype. The resulting expressed Cry3Bb1 protein shares 99.1% sequence identity with wildtype Cry3Bb1. Further, the amino acid sequence of MON 88017 differs by only 1 of the 653 amino acids and therefore shares 99.8% identity to previously deregulated line MON 863 (Petition #01-137-01p). The introduced *cp4 epsps* gene encodes for tolerance to glyphosate, thereby allowing for selection of transformed plants in the presence of glyphosate.

Southern blot analysis was performed on multiple generations of MON 88017 corn plants to determine the stability of inserted DNA. The breeding tree for MON 88017 is presented in Figure V-12 (page 70 of Petition) and describes plant samples used in stability experiments. MON 88017 and control genomic DNA was digested with *Xba* I, which digests once in PV-ZMIR39. The blot was probed simultaneously with four radiolabeled probes (Probes 1 – 4, Figure V-1a) that span the entire length of the insert. Lane 5 (previously characterized generation) and lanes 6 – 11 of the Southern blot in Figure 13 (page 71 of Petition) show DNA bands of the same molecular weight indicating stability of the insert over several generations.

Chi square analysis was performed to confirm Mendelian inheritance of the corn rootworm resistance and glyphosate tolerance traits in MON 88017. Data from 10 generations of MON 88017 corn were tested and identified as positive for corn rootworm protection based upon ELISA results positive for the Cry3Bb1 protein. The glyphosate tolerance trait was deduced from the data for the Cry3Bb1 protein based upon the linkage of the *cp4 epsps* and *cry3Bb1*genes in the PV-ZMIR39 vector and the expected identical segregation ratios between these genes in MON 88017 progeny. Results of the Mendelian inheritance analysis are provided in Table V-1 (page 76 of Petition) and indicate that all but two values showed no significant differences between the observed and expected frequency in eight breeding generations. The results for generation LH198BC1F_1 were significant at $p \leq 0.05$, but not at $p \leq 0.01$. The results for LH198BC0F_1 x LH59 were the result of gamete selection caused by Roundup® according to the developer. Gamete selection was previously observed for Roundup Ready corn (Petition #00-011-01p). Despite the deviation seen in these two generations, when the Chi-square test was performed across all generations, the expected 3:1 segregation ratio is obtained, consistent with a dominant trait at a single locus for MON 88017 corn.

The initial germplasm designated as transformation event MON 88017 was used in a breeding program to produce commercially viable hybrid corn lines with the newly introduced traits. APHIS analyzed data and information submitted in the petition that characterized the nature, stability, inheritance, and expression of the inserted genetic constructs and their encoded proteins in different generations of plants derived from MON 88017. DNA analysis of corn grains supports the conclusion that: (1) MON 88017 corn contains within its genomic DNA (nuclear chromosomes) a single copy of the intact gene cassette that contains both the *cry3Bb1* and *cp4 epsps* genes and their associated noncoding regulatory regions; and (2) these genetic constructs were stably and predictably inherited according to Mendel's laws over seven generations of cross-fertilizations and three generations self-pollinations. Analyses using Southern blots indicate a single insertion site, intact expression cassettes, and single copy numbers of both the *cry3Bb1* and the *cp4 epsps* genes. Data in the petition support the conclusion that hybrids derived from line MON 88017 exhibit the expected trait of corn rootworm control conferred by the production of the insecticidal protein, Cry3Bb1 and glyphosate tolerance conferred by the production of the CP4 EPSPS enzyme.

Phenotypic data was submitted to APHIS from field tests conducted at 8 field sites in 2001 and 10 field sites in 2002 (Table VII-2, page 93 of Petition). Within-site analysis for 2001 field trials identified only four statistical differences in 100 comparisons (4%): two for ear height and grain moisture, one for early stand count and one for staygreen. These differences occurred at single sites indicating likely random effects. The across-site analysis identified only one statistically significant difference for seedling vigor (Table VII-3, page 97 of Petition). Test seedlings were more vigorous than control seedlings, although there were no across-site differences in stand count, days to pollen shed or days to silk emergence and therefore, the difference in seedling vigor does not appear to be a significant variation. There were no differences in lodging, dropped ears, insect or disease susceptibility, or responses to biotic and abiotic stressors (Table VII-4, page 98 of Petition)

Within-site analysis for 2002 showed statistical differences in 11 of 140 comparisons (7.9%). Seedling vigor was significantly greater for test seedlings at three of ten sites; early stand count for test plants were higher at two sites and lower at one site; 50% pollen shed occurred one day earlier for test plants at one site; ear height was lower at one site for test plants; plant height was lower at one site for test plants; fewer stalk lodged plants were detected at one site for test plants; and yield was higher at one site for test plants. The across-site analysis showed two significant differences: seedling vigor was greater for test plants and days to 50% pollen shed was slightly shorter for test plants. Neither of these across-site differences appear to be biologically significant. Table VII-6 (page 102 of Petition) lists the results of disease and insect susceptibility, as well as susceptibility to abiotic stressors. There were small qualitative differences between test and control plants for corn rootworm, anthracnose and chemical injury incidence at single sites, but none of these differences appear to increase the pest potential of MON 88017 corn.

In addition to field studies on agronomic parameters, Monsanto analyzed grain of MON 88017 corn for compositional changes as a part of their submission to FDA in the consultation process. While FDA uses these data as an indicator of nutritional changes, APHIS views them as a general indicator of unintended changes. MON 88017corn is compared to a non-transgenic control and to the commercial range for components such as minerals, vitamins, fatty acids, carbohydrate, protein, and fat (Table VII-8, page 109 of Petition). The data indicate no changes in 232 of the 248 comparisons performed. All of the statistically significant test values were within the 99% tolerance interval. These results provide additional evidence that MON 88017corn will not exhibit unexpected and unintended effects.

B. Potential impacts based on the relative weediness of MON 863 corn.

APHIS assessed whether MON 88017 corn is any more likely to become a weed than the nontransgenic recipient corn line, or other corn currently cultivated. The assessment encompasses a thorough consideration of the basic biology of corn and an evaluation of unique characteristics of MON 88017 corn.

In the United States, 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 evaluated seeds from MON 88017 corn to determine if there was any change in dormancy or germination, as these could indicate a change in weediness (see Petition, section VII.A.3). Phenotypic data collected in field tests conducted at 18 locations in 2001 and eight locations in 2002 from Nebraska, Illinois, Missouri, Indiana and Iowa were used to assess the enhanced weed potential of the modified crop. These data resulted in no differences in lodging or dropped ears between MON 88017 plants and their non-transgenic counterparts demonstrating a lack of enhanced weed potential. Extensive postharvest monitoring for volunteers of these field trials conducted under USDA-APHIS notifications did not reveal any differences in survivability or persistence of MON 88017 relative to other corn. In addition, field tests conducted with MON 863 in 2000 from multiple sites in Illinois, Iowa and Nebraska resulted in no observed differences between six MON 863 and non-transgenic hybrids in characteristics which might increase the plants ability to compete or persist as a weed.

Seed dormancy is an important characteristic that is often associated with plants that are weeds (Anderson 1996). Growth chamber studies were conducted according to the Association of Official Seed Analysis (AOSA 1998) standards to evaluate seed dormancy and germination characteristics including percent normal germinated seed, percent viable hard seed, percent abnormal germinated seed, percent viable firm swollen seed and percent dead seed. Dormancy mechanisms, including hard seed, vary with species and tend to involve complex processes. A comparison of MON 88017 seeds to a non-transgenic control and four conventional reference corn hybrids resulted in no biologically significant differences in seed dormancy or germination characteristics at optimal growing temperatures (see Petition, Appendix E for details and data tables). From these studies, it can be concluded that MON 88017 corn does not exhibit any characteristics that would cause it to be more weedy than the parent corn line.

Changes in pollen morphology might indicate an increased ability for pollen to travel or cross-pollinate other corn plants that may result in increased weediness. Pollen morphology characteristics including diameter and viability were evaluated from a field trial conducted in Illinois in 2003 (USDA notification 03-112-14n). The overall morphology and pollen viability were similar among MON 88017 and non-transgenic corn (see Petition, section VII.A.7, Table VII-7, Figures VII-1 and VII-2). Although there was a small increase in MON 88017 pollen viability relative to its non-transgenic counterpart, this difference was not large enough to be considered biologically significant. These results demonstrated that there are not changes in pollen morphology and viability of MON 88017 corn that would lead to an enhanced weed potential.

The *cry 3Bb1 gene* introduced for coleopteran insect resistance and *cp4 epsps* gene introduced for glyphosate tolerance are not expected to cause MON 88017 corn to become a weed. None of the characteristics of weeds described by Baker (1965) involve resistance or susceptibility to insects, and there is no reason to expect that the protection against the target insects provided by this new corn line would release it from any constraint that would result in increased weediness. The CP4 EPSPS protein produced in MON 88017 corn is similar to the native EPSPS proteins that are ubiquitous in plant and microbial tissues in the environment. Based on its history of occurrence and laboratory investigations with arthropods potentially exposed to Roundup Ready crops (Goldstein 2003, Boongird *et al.* 2003, Jamornman *et al*. 2003, Harvey *et al.* 2003), the EPSPS protein is not expected to possess biological activity towards non-pest organisms.

C. Potential impacts from gene introgression from MON 88017 corn into its sexually-compatible relatives.

APHIS evaluated the potential for gene introgression to occur from MON 88017 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*.

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

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* ssp *mexicana* forms frequent hybrids with maize, *Z. luxurians* hybridizes only rarely with maize, whereas populations of *Z. mays* ssp. *parviglumis* are variable in this regard (Wilkes 1977, Doebley 1990a). Research on sympatric populations of maize and teosinte suggests introgression has occurred in the past, in particular from maize to *Z. mays* ssp. *luxurians* and *Z. mays* ssp. *diploperennis* and from annual Mexican plateau teosinte (*Z. mays* ssp. *mexicana*) to maize (Kato Y. 1997 and references therein).

Nonetheless, in the wild, introgressive hybridization from maize to teosinte is currently limited, in part, by several factors including distribution, differing degrees of genetic incompatibility, differences in flowering time in some cases, block inheritance, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Doebley 1990a and 1990b; Galinat 1988). First-generation hybrids are generally less fit for survival and dissemination in the wild, and show substantially reduced reproductive capacity which acts as a significant constraint on introgression. Teosinte has coexisted and co-evolved in close proximity to maize in the Americas over thousands of years, but maize and teosinte maintain distinct genetic constitutions despite sporadic introgression (Doebley 1990a). Gene introgression from MON 88017 corn into teosinte would require that varieties be developed, and approved for cultivation in locations where these teosintes are located. Since MON 88017 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 varieties of cultivated corn.

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 88017 corn under natural conditions is highly unlikely or impossible. Hybrids of *Tripsacum* species with *Zea* are difficult to obtain outside of a laboratory and are often sterile or have greatly reduced fertility, and none are able to withstand even the mildest winters. Furthermore, none of the sexually compatible relatives of corn in the U.S. are considered to be weeds in the U.S. (Holm et al. 1979), therefore, the unlikely acquisition of a single pesticide gene would not be expected to transform them into a weeds.

D. Potential impact on nontarget organisms, including beneficial organisms and threatened or endangered species.

Potential Impact on Non-target Species
Like the Cry1 class of insecticidal proteins, the specificity of the Cry3Bb1 insecticidal activity is dependent upon their binding to specific receptors present in the insect mid-gut (Lambert, et al., 1996; Van Rie et al., 1990; Van Rie et al., 1989; Hoffmann et al., 1988a and 1988b; and Wolfersberger et al., 1986). The Cry3Bb1 protein expressed in MON 88017 corn has activity only against select beetle (Order Coleoptera) species within the family Chrysomelidae, namely CRW and Colorado potato beetle. Monsanto conducted a series of diet bioassays to characterize the insecticidal specificity of Cry3Bb1 (see Petition Section VIII.B.1). Test species were: Colorado potato beetle, western corn rootworm, cowpea weevil, red flower beetle, cotton boll weevil, pepper weevil, rice weevil, corn earworm, and the European corn borer. These bioassays confirmed the high specificity of Cry3Bb within the Order Coleoptera.

Likewise, the Cry3Bb1 protein is not expected to adversely affect most other invertebrates and all vertebrate organisms, including non-target birds, mammals and humans, because they would not be expected to contain the receptor protein found in the midgut of target insects. To evaluate the potential of MON 88017 corn to have damaging or toxic effects on representative terrestrial and an aquatic species, APHIS evaluated data from a series of ecological toxicology experiments (see Petition, section VIII.B.2 and Table VIII-1). The test organisms included bobwhite quail as a representative avian species and channel catfish as a representative fish. Several beneficial invertebrates were also evaluated including predators (adult and larval lady beetles of the species, *Coleomegilla maculata*, adult lady beetles of the species *Hippodamia convergens* and green lacewing larvae), a representative parasitic wasp (*Nasonia vitripennis*), decomposing organisms (Collembola, and earthworms), and aquatic invertebrate (waterfleas, *Daphnia magna*) and adult and larval honey bees. These non-target organisms were fed one of the following: leaf tissue, grain or pollen containing plant-produced Cry3Bb1 protein; or an artificial diet containing microbially-produced protein; or a recombinant Bt strain expressing the protein from the same variant gene used in MON 88017 corn. No adverse effects were observed at the maximum concentrations to which the various test organisms would be exposed to in corn (see Appendix C of this EA).

In addition to laboratory dietary toxicity studies, a two-year field study was conducted with event MON 863 corn expressing the Cry3Bb1 protein at similar levels as MON 88017 corn. For this study, field data was collected to assess possible effects of growing corn containing the Cry3Bb1 protein on the relative abundance of certain beneficial insects. Soil dwelling invertebrates (Diplura (Japygidae), Chilopoda, Aranea, Acari, Oligocaeta (earthworms), ants (Formicidae) and Coleoptera including Carabidae, Staphylinidae, Nitidulidae and Lanthridiidae) important in decomposition of plant litter were collected from root and soil samples. Predatory (Carabidae, Staphylinidae, ants (Formicidae), spiders and centipedes) and decomposing (Gryllidae, Nitidulidae) ground surface-dwelling invertebrates were collected in pitfall traps. Yellow sticky traps were used to sample highly mobile and foliage-dwelling invertebrates including predators (Coccinellidae, Anthocoridae, Chrysopidae, Hemerobiidae and spiders), decomposers (Nitidulidae and Syrphidae) and parasitic Hymenoptera. Results indicated that field collected beneficial invertebrates were at least as abundant in MON 863 plots as in the control plots indicating no impact from the introduction of the *cry3Bb1* gene. For many insects, MON 863 corn had a lesser impact than did conventional pest management schemes involving chemical insecticides. Due to their similarity, MON 88017 corn is expected to have comparable impacts on non-target organisms as MON 863.

The glufosinate herbicide tolerant *cp4 epsps* gene and the regulatory elements also do not pose a significant risk to non-target organisms. The *epsps* gene has a long history of safe use and has no known toxicity to non-target organisms. The various regulatory elements are not expressed in the plant, and therefore there is no reason to believe that deleterious effects or significant impacts would result from their use.

Appendix C of this environmental assessment is a summary table in which the Cry3Bb1 protein expressed in MON 88017 corn is compared to conventional chemical insecticides used to control corn rootworms. The comparison encompasses environmental fate and potential nontarget effects. In general, MON 88017 compares favorably to these products with respect to the potential for harm in the environment. In addition, Monsanto's studies indicate that Cry3Bb1 protein is readily degraded in simulated gastric fluid and simulated intestinal fluid and is therefore not likely to be an allergen (see petition Appendix C, Section 9). Accordingly, EPA has ruled that there is reasonable certainty of no harm from consumption of the Cry3Bb1 protein and that it is not considered an allergen (66 *FR* 24061-24066, May 11, 2001). For this reason, allergic reactions in non-target species are considered to be highly unlikely.

Potential Impact on threatened and endangered species

APHIS coordinates review of petitions with other agencies that have regulatory oversight on that same product. With respect to threatened and endangered species, EPA plays a leadership role in the evaluation. EPA evaluated the Cry3Bb1 protein for Monsanto's application for registration of MON 863 corn. Given the specificity of the Cry3Bb1 activity, species outside the insect order Coleoptera should not be affected. EPA thoroughly examined all threatened and endangered beetles that occur in counties where corn is grown, and determined that most occur in cave or aquatic habitats. None of the endangered beetles are expected to occur or breed in cornfields. The American burying beetle was given particular consideration since it may occur in old fields or cropland hedge rows. However, the American burying beetle is not expected to occur within cornfields nor will it be exposed to Cry3Bb1 protein since they are opportunistic scavengers that only feed on dead vertebrate carcasses buried under the soil surface.

E. Potential impacts on agricultural and cultivation practices

APHIS considered potential impacts associated with the cultivation of rootworm-resistant and glyphosate tolerant MON 88017 corn on current agricultural practices, in particular, those currently associated with corn rootworm and weed control in corn. The potential impact on organic farming and on minorities and children were also considered.

Potential impacts of line DAS-59122-7 corn on insect control practices

Monsanto has provided data which indicate that MON 88017 corn expresses the modified Cry 3Bb1 protein in root tissues to provide control of corn rootworms. The availability of this product is likely to have an impact on current control practices for corn rootworm that include the use of crop rotation, chemical insecticides, and other Bt corn varieties intended to control corn rootworm. Both crop rotation and the use of chemical insecticides have been important strategies in the past, with commercial use of the YieldGard® Rootworm variety increasing in the past couple of years since deregulation. However, CRW have developed several adaptations to control methods including crop rotation and insecticide resistance. Since CRW predominantly oviposit in corn fields, rotating corn with small grains, hay, clover or alfalfa has been utilized as a control method (Levine and Oloumi-Sadeghi 1991). CRW have also been controlled by planting soybean after corn since CRW cannot survive on soybean. Rotating soybeans after corn decreases the need for CRW-targeted insecticide applications. However, WCRW has developed an adaptation to resist the corn/soybean rotation in Illinois and Indiana (Levine and Oloumi-Sadeghi 1996). In areas such as east-central Illinois and northern Indiana, the WCRW has been found to have the ability to lay eggs in soybean, overwinter and hatch the following year in corn (Levine and Oloumi-Sadeghi 1991, Levine and Oloumi-Sadeghi 1996, O'Neal *et al.* 1999, Isard *et al.* 1999, Isard *et al.* 2000).

Northern CRW populations have also developed resistance to the corn/soybean rotation in Minnesota, Iowa, and South Dakota (Gray *et al.* 1998). Prolonged diapause of NCRW involves eggs that remain viable for two winters and hatch two seasons after being laid. Northern CRW have developed the ability for prolonged or extended diapause resulting in a significant proportion of their eggs hatching after two winters leading to an adaptation to rotating corn with crops such as soybean. Extended diapause has been verified in the laboratory from NCRW eggs collected from South Dakota, Minnesota, Illinois and Michigan (Krysan *et al*. 1984, Krysan *et al* 1986, Levine and Oloumi-Sadeghi 1991 Levine *et al*. 1992a and 1992b). Field studies conducted by Tollefson (1988) in northwestern Iowa corn fields suggest that extended diapause occurs throughout NCRW distribution in rotated fields. Another study conducted by Levine and Oloumi-Sadeghi (1996) suggests that the WCRW does not demonstrate extended diapause.

In addition to the problem with insect adaptation to crop rotation, many growers simply prefer to grow corn continuously, a practice which necessitates higher inputs of chemical insecticides. As a result of these factors and the very damaging nature of the pest, chemical insecticide usage has increased. The most common chemical regime is the application of a granular insecticide at planting, either banded or in-furrow. In some cases sprays are applied for adult suppression.

Instances of CRW resistance to crop rotation and/or insecticide use typically develop on a local scale which is probably due to limited adult movement before and after mating. In these cases, resistance took at least ten and usually more than 15 years to develop without implementing IRM strategies. Research is currently underway at the University of Nebraska and USDA-ARS in North Dakota to determine the genetics of esterase-mediated insecticide resistance in WCRW populations. Results of this research are intended to provide knowledge on localized selection and migration that may aid in refining future IRM strategies.

A risk and benefits assessment for re-registration of Bt corn and cotton plant incorporated protectants (PIP's) has been prepared by the EPA (U.S. EPA, 2000) and is posted at the following EPA internet site: http://www.epa.gov/scipoly/sap. Issues considered by the EPA pertaining to this assessment were the subject of a meeting convened on October 18-20, 2000 by the EPA Federal Scientific Advisory Panel (SAP). EPA also convened a SAP meeting, August 27-29, 2002, to consider issues related to corn rootworm-related PIP's. The results of this SAP meeting can be found at: http://www.epa.gov/scipoly/sap/2002/index.htm. Before these new Bt corn varieties were available, farmers were willing to accept lower corn yields, rather than incur the expense, trouble, and uncertain results of chemical insecticide applications to control the target pests. Following the registration of Bt corn varieties in 1995, growers were quick to embrace the new technology. Estimates of Bt corn acreage as a percent of total corn acreage planted increased from 1% in 1996 to 26% in 1999, 2000, and 2002 (USDA NASS, and http://www.usda.gov/nass/pubs/bioc0703.pdf). The USDA National Agricultural extension Service (NASS) statistics compiled from 15 top corn producing states in the Midwest indicate that 30% of this acreage were treated with insecticide registered for corn rootworm control. It is difficult to surmise how much of this application was for the corn rootworm control as these insecticide products used alone or in combination also control other pests such as black cutworms. A 1995 survey conducted in Iowa, the leading corn producing state which accounts for 17.5% of all U.S. production, indicated that growers used chemicals to control CRW 22 % of the time. The most widely used insecticides are from the organophosphate or synthetic pyrethroid classes of insecticides. It is therefore expected that availability of a another practical and economical alternative to chemical insecticides for CRW control would result in a significant reduction in application of such chemicals.

MON 88017 corn could be incorporated into current integrated pest management practices as an additional tool for control. Fields are typically scouted for adult CRW in the late summer or early fall. Economic thresholds are then used in making decisions about control strategies for the following spring planting season. MON 88017 offers an alternative to organophosphate and pyrethroid insecticide applications in cases where thresholds indicate CRW control is needed and the grower chooses to grow corn. No new or specialized equipment or skills would be needed to use the new technology. Reduced pesticide usage by the growers would carry the accompanied benefits of reduced needs for the manufacture, transport, storage and disposal of hazardous chemicals and containers.

Cry3Bb1-resistant populations of previously sensitive insects may eventually develop as a result of feeding on MON 88017 corn plants. Monsanto acknowledges this in the petition and is conducting research and implementing insect resistant management strategies (IRM) for corn containing Cry3Bb1 that have been approved by the EPA. These strategies were developed in cooperation with experts in government and from academia. IRM requirements for MON 863 corn would also be adopted for MON 88017 corn and include a 20% non-Bt corn refuge planted adjacent to or within the Bt cornfields. Refuges planted as row strips within a cornfield include at least 6, and preferably 12 consecutive rows. Refuge acres may be treated to control CRW larvae with chemical insecticides, but insecticides should not be used on refuges to control CRW adults. Alternate hosts and seed mixes are not acceptable refuges MON 88017 corn. If resistance to Cry3Bb were to develop, it would not alter the effectiveness of current agricultural practices used to control CRW.

Impacts of previously deregulated herbicide tolerant corn on weed control
Several herbicide tolerant corn varieties are commercially available. These were described under Alternative A. The first glufosinate-ammonium tolerant corn varieties were deregulated by APHIS in June 1995. In 1996, prior to the introduction of Roundup Ready (glyphosate herbicide tolerant) corn, pest management data for corn indicate that: 1) 3% of acres planted were to herbicide resistant varieties; 2) 83% of pesticide treatments were for weed control, and of those, 20% were post emergence, 39% pre-emergence, and 41% both; 3) mechanical cultivation was used for weed control on 51% of acres planted (Fernandez-Cornejo and Jans 1999). It is estimated that the adoption of other herbicide tolerant corn varieties was associated with an overall decrease in herbicide use in 1996 (especially for the chloroacetamide herbicide family) (Fernandez-Cornejo and McBride 2000). Nonetheless, in 1997, 96% of the corn acreage in the 10 major corn-producing states was treated with herbicides. At least 18 different herbicide active ingredients have been used, many in combination. Atrazine (which performs well for control of broadleaf weeds) and the chloroacetamides, metolachlor and acetochlor (which perform well for control of annual grass weeds) together accounted for 72% of the total applied in 1997 (Knake 1998, Fernandez-Cornejo and McBride 2000). Overall herbicide use has remained relatively consistent since 1999, with the exception of an increase in glyphosate and mesotrione use and a decrease in metolachlor use (Appendix E). Glyphosate use in corn has increased from 9% in 1999 to 19% in 2003 (NASS, 2000; NASS, 2004). This is consistent with the increased adoption of herbicide-tolerant corn. Herbicide-tolerant corn was planted on 14% of corn acreage in 2003, representing a 75% increase in herbicide-tolerant corn acreage compared to 2001 (Sankula and Blumenthal 2004). Glufosinate use on corn has remained relatively low since 1999, with use on 2% of corn acreage in 1999 and use on about 3% of corn acreage in 2003 (NASS, 2004; NASS, 2000).

Potential impacts of line DAS-59122-7 corn on weed control
APHIS evaluated data submitted by the petitioners that show that hybrids derived from MON 88017 corn express CP4 EPSPS that provides the corn with tolerance to glyphosate herbicides. MON 88017 corn, along with glyphosate herbicides, is expected to positively impact current agricultural practices used for weed control in a manner similar to other previously deregulated glyphosate tolerant corn, that is by 1) offering growers a broad spectrum, post-emergent weed

control system for both broadleaf and grass weeds; 2) providing the opportunity to continue to move away from pre-emergent herbicides such as metachlor; 3) providing an alternative herbicidal mode of action in corn that allows for improved management of weeds in corn that have developed resistance to herbicides with different modes of action, e.g. triazines and acetolactate synthase (ALS) inhibitors (see *http://www.weedscience.org/Resistance/situation.asp*); and 4) decreasing cultivation needs and increasing the number of no-till acres.

Volunteers of MON 88017 corn, can be controlled by selective mechanical or manual weed removal or by the use of certain herbicides with active ingredients other than glyphosate. 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). Glufosinate-ammonium herbicides could also be used to control glyphosate tolerant volunteers of MON 88017 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). 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).

APHIS considered the possibility that availability and use of glyphosate tolerant corn lines such as MON 88017 corn could lead to greater use of glyphosate herbicide and result in selection and establishment of weeds tolerant to this herbicide. This would have herbicide use implications both for use of glyphosate tolerant crops previously deregulated by APHIS and possibly for other crops grown in rotation. The occurrence of weeds tolerant to other herbicides is well documented, and technical assistance is available to help identify, prevent, and mitigate this risk (Heap 2000). The risk of glyphosate tolerant weeds developing appears to be quite low. While all herbicides have varying degrees of effectiveness against different weeds, a worldwide survey of herbicide resistant weeds lists seven weed species (as of April 1, 2005) with glyphosate resistance worldwide, of which two are in the United States (http://www.weedscience.org/in.asp). Current practices involving rotation of herbicides with different modes of action and cultivation or mowing to eliminate weeds should be effective in reducing or managing the risk. Because of the lack of cross-resistance between glufosinate-ammonium and glyphosate, MON 88017 corn could provide an additional alternative for crop rotation. APHIS and the EPA Herbicide Division (Registration Division; Herbicides Branch) have initiated a working group to ensure thorough ongoing considerations of issues surrounding herbicide resistant plants, including the potential for the development of glyphosate tolerant weeds.

Potential impacts on organic farming

It is not likely that organic farmers, or other farmers who choose not to plant transgenic varieties or sell transgenic grain, will be significantly impacted by the expected commercial use of this product since: (a) non-transgenic 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 Cry3Bb coleopteran resistant and glyphosate-tolerant; and (c) based on the IRM plan, farmers will be educated about recommended management practices. Several transgenic corn lines resistant to lepidopteran insects are already in widespread use by farmers. Transgenic herbicide tolerant varieties are also available. This particular product should not present new and different issues than those with respect to impacts on organic farmers. APHIS has considered that corn is open-pollinating and it is possible that the engineered genes could move via wind-blown pollen to an adjacent field. All corn, whether genetically engineered or not, can transmit pollen to nearby fields, and a very small influx of pollen originating from a given corn variety does not appreciably change the characteristics of corn in adjacent fields. As described previously in this assessment, the rate of cross-pollination from one field to another is expected to be quite low, even if flowering times coincide. The frequency of such an occurrence decreases with increasing distance from the pollen source such that it sufficiently low at 660 feet away to be considered adequate for production of certified corn seeds.

F. Potential impacts on raw or processed agricultural commodities.

Our analysis of data on agronomic performance, disease and insect susceptibility, and compositional profiles of the kernels indicate no differences between MON 88017 and their non-transgenic hybrid counterparts or other standard hybrids. APHIS does not foresee either a direct or indirect plant pest effect on any raw or processed plant commodity.

G. Cumulative Impacts

APHIS considered whether the proposed action could lead to cumulatively significant impacts, when considered in light of other past, present, and reasonably foreseeable future actions, regardless of what agency or person undertakes such actions. In the preceding analysis we have considered the potential for stacking of multiple herbicide tolerance genes, from MON 88017 corn and other herbicide tolerance genes in previously deregulated transgenic corn lines or in corn developed by other methods, to pose a weed management problem. We have also considered the cumulative impacts of non-transgenic and previously deregulated transgenic herbicide tolerant corn, and other herbicide tolerant crops typically grown in rotation with corn, on the type and toxicity of herbicides and other management practices that can be used to manage weeds in these crops, including the development and management of herbicide tolerant weeds. We have reviewed and considered studies and reports (e.g. U.S. EPA, 2000a; Fernandez-Cornejo and Mcbride, 2000; USDA-ARS, 2003; Sankula and Blumenthal, 2004) to predict the cumulative impacts of deregulation and any subsequent registration and commercialization of another Cry 3Bb1 corn, in light of other transgenic coleopteran-resistant Bt plants currently on the market, and the potential for stacking with different lepidopteran resistance genes. The risk assessment included evaluating potential impacts on non-target organisms, changes in pesticides used to control the target pests and other non-targets pests, and the potential for resistance of the Bt toxins to develop as a result of exposure to these toxins in Bt PIPs or in other Bt formulations.

Alternative C, Approval of the Petition in Part

Approval of some but not all of lines requested in the petition. The petition requested a determination of non-regulated status only for lines derived from the one transformation event, designated as MON 88017. Therefore, APHIS can consider only that one line for approval.

Approval of the petition with geographic restrictions. EPA is currently reviewing the application to register MON 88017 corn as a plant pesticide. EPA has completed a thorough analysis of risks to non-target organisms and to threatened and endangered species. After examining all threatened and endangered beetles that occur in counties where corn is grown, they have concluded that none of the beetles= breeding habitats are shared with corn. Based on this finding, APHIS finds no reason to place geographic restriction on planting of MON 88017 corn.

VI. CONSIDERATION OF EXECUTIVE ORDERS, STANDARDS AND TREATIES RELATING TO ENVIRONMENTAL IMPACTS

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

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

Each alternative was analyzed with respect to EO 12898 and 13045. None of the alternatives are expected to have a disproportionate adverse effect on minorities, low-income populations, or children. Collectively, the available mammalian toxicity, along with the history of safe use of microbial Bt products and other corn varieties expressing Bt proteins and CP4 EPSPS, establishes the safety of MON 88017 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 88017 corn described above are expected to have a disproportionate adverse effect on minorities, low income populations, or children. As noted above, the cultivation of previously deregulated corn varieties with similar insect resistance and herbicide tolerance 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.

Potential impacts on invasive species
EO 13112, "Invasive Species", states that federal agencies take action to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause. Non-engineered corn as well as other Bt and herbicide tolerant corn varieties are widely grown in the United States. 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.

Potential environmental impacts outside the United States associated with a determination of nonregulated status as requested by Monsanto
Executive Order 12114, "Environmental Effects Abroad of Major Federal Actions" requires Federal officials to take into consideration any potential environmental effects outside the U.S., its territories and possessions that result from actions being taken. APHIS has given this due consideration and does not expect a significant environmental impact outside the United States should nonregulated status be determined for MON 88017 corn or if the other alternatives are chosen. It should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new corn cultivars internationally, apply equally to those covered by an APHIS determination of nonregulated status under 7 CFR Part 340. Any international traffic in MON 88017 corn subsequent to a determination of non-regulated status for line MON 88017 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." The protection it affords extends to natural flora and plant products and includes both direct and indirect damage by pests, including weeds. The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (116 countries as of June, 2001). In April, 2004, a standard for pest risk analysis of living modified organisms (LMOs) was adopted at a meeting of the governing body of the IPPC as a supplement to an existing standard, International Standard for Phytosanitary Measure No. 11 (ISPM-11; Pest Risk Analysis for Quarantine Pests). The standard acknowledges that all LMOs will not present a pest risk, and that a determination needs to be made early in the PRA for importation as to whether the LMO poses a potential pest risk resulting from the genetic modification. APHIS pest risk assessment procedures for bioengineered organisms are consistent with the guidance developed under the IPPC. In addition, issues that may relate to commercialization and transboundary movement of particular agricultural commodities produced through biotechnology are being addressed in other international forums and through national regulations.

The Cartagena Protocol on Biosafety is a treaty under the United Nations Convention on Biological Diversity (CBD) that established a framework for the safe transboundary movement, with respect to the environment and biodiversity, of LMOs, which includes those modified through biotechnology. The Protocol came into force on September 11, 2003 and 119 countries are parties to it as of April 14, 2005 (see http://www.biodiv.org/biosafety/default.aspx). Although the United States is not a party to the CBD, and thus not a party to the Cartagena Protocol on Biosafety, US 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). This 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 United States and in the Organization for Economic Cooperation and Development. 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. USDA Approved Field Tests with Bt *cry3Bb1* Corn Line MON 88017 Listed by Notification Number.

2000-2001 Field Trials
99-342-02n
00-082-10n
00-090-01n
00-090-02n
00-090-03n

2001-2002 Field Trials
00-339-02n
00-348-02n
00-356-11n
01-019-11n
01-022-05n
01-022-08n
01-022-09n
01-022-12n
01-022-14n
01-023-08n
01-023-09n
01-023-10n
01-023-11n
01-023-12n
01-023-15n
01-023-16n
01-023-17n
01-024-07n
01-026-17n
01-026-18n
01-026-19n
01-026-20n
01-043-04n
01-051-26n
01-127-02n
01-197-05n
01-197-06n
01-197-07n
01-242-02n
01-256-04n
01-354-04n

2002 Field Trials
02-007-02n
02-007-03n
02-007-04n
02-007-05n
02-007-07n
02-008-07n
02-008-13n
02-008-14n
02-008-24n
02-008-25n
02-008-26n
02-008-27n
02-008-28n
02-009-01n
02-009-02n
02-009-03n
02-009-04n
02-009-06n
02-009-07n
02-009-08n
02-010-12n
02-010-13n
02-010-14n
02-010-15n
02-010-17n
02-014-04n
02-014-05n
02-014-06n
02-014-07n
02-014-08n
02-014-09n
02-014-10n
02-014-11n
02-014-12n
02-015-01n
02-015-02n
02-015-03n
02-022-17n
02-022-27n
02-022-29n
02-022-30n
02-022-31n
02-022-32n
02-022-33n
02-022-34n
02-022-35n
02-022-36n
02-022-47n
02-022-48n
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02-022-52n
02-023-02n
02-023-03n
02-023-04n
02-023-05n
02-023-06n
02-023-07n
02-023-09n
02-023-10n
02-028-11n
02-028-12n
02-028-13n
02-028-14n
02-028-15n
02-028-16n
02-028-17n
02-028-18n
02-028-19n
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02-028-21n
02-030-02n
02-030-03n
02-031-03n
02-031-04n
02-031-05n
02-031-06n
02-031-07n
02-036-03n
02-036-04n
02-036-05n
02-036-06n
02-036-09n
02-036-15n
02-036-17n
02-037-04n
02-037-07n
02-037-08n
02-042-17n
02-042-18n
02-043-04n
02-051-18n
02-066-14n
02-070-27n
02-092-07n
02-092-08n

02-115-04n
02-213-05n
02-213-08n
02-214-04n
02-247-05n
02-309-04n

2003 Field Trials

03-015-02n
03-015-03n
03-015-04n
03-015-05n
03-015-06n
03-015-11n
03-021-08n
03-021-09n
03-021-10n
03-021-11n
03-021-12n
03-021-13n
03-022-02n
03-022-05n
03-023-12n
03-027-05n
03-030-04n
03-030-08n
03-030-09n
03-030-13n
03-030-14n
03-030-15n
03-030-17n
03-030-18n
03-034-12n
03-034-13n
03-034-14n
03-034-15n
03-034-16n
03-034-17n
03-034-18n
03-034-19n
03-034-20n
03-034-21n
03-034-22n
03-034-23n
03-034-29n
03-034-31n
03-034-32n
03-035-01n
03-035-02n
03-035-03n
03-035-04n
03-035-05n
03-035-06n
03-035-07n
03-035-08n
03-037-01n
03-037-02n
03-037-03n
03-037-04n
03-037-05n
03-037-09n
03-042-08n
03-042-16n
03-042-18n
03-043-01n
03-043-03n
03-083-01n
03-098-05n
03-112-04n
03-112-08n
03-112-09n
03-112-10n
03-112-14n
03-184-02n
03-219-05n
03-230-01n
03-259-02n
03-279-02n
03-266-02n
03-272-06n
03-301-09n
03-301-10n
03-301-11n
03-301-12n
03-301-13n
03-301-14n
03-322-02n
03-322-03n
03-356-01n
03-356-02n
03-356-04n

2004 Field Trials

04-006-03n
04-014-06n
04-021-04n
04-021-06n
04-021-07n
04-021-08n
04-022-01n
04-022-02n
04-023-01n
04-023-02n
04-023-03n
04-023-05n
04-023-06n
04-023-07n
04-023-08n
04-028-11n
04-028-12n
04-028-13n
04-028-14n
04-028-15n
04-028-16n
04-028-18n
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04-028-21n
04-028-22n
04-028-34n
04-028-35n
04-028-47n
04-028-48n
04-028-49n
04-028-50n
04-028-51n
04-028-52n
04-030-03n
04-035-03n
04-035-04n
04-035-05n
04-036-05n
04-037-04n
04-037-05n
04-037-06n
04-037-07n
04-037-08n
04-037-09n
04-037-10n
04-037-11n
04-041-06n
04-041-07n
04-041-08n
04-042-15n
04-048-05n
04-055-05n

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

Wild diploid and tetraploid members of *Zea* collectively referred to as teosinte are normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua. A few isolated populations of annual and perennial teosinte have been reported to exist in Florida and Texas, respectively (USDA-APHIS, 1998b); but local botanists and agronomists familiar with the flora of these regions have not documented any current populations of teosinte there (U.S. EPA, 2000a, see page IIC5). The Mexican and Central America teosinte populations primarily exist within and around cultivated maize fields; they are partially dependent on agricultural niches or open habitats, and in some cases are grazed upon or fed to cattle which distribute the seed. While some teosinte may be considered to be weeds in certain instances, they are also used by some farmers for breeding improved maize (Sánchez and Ruiz, 1997, and references therein).

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

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

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

Appendix C. Table of Products Used or Proposed for Use in Corn Rootworm Control. Cry3Bb1 is expressed in tissues of Monsanto's corn lines.

	Cry34/35Ab1	Cry3Bb1	Terbufos (Counter®)	Tefluthrin (Force®)
Environmental Fate	The DT_{50} estimate for Cry34/35Ab1 binary protein in soil was found to be 3.2 days. (1)	The maximum environmental concentration for organisms feeding on corn plants is predicted to be 93 ug/g based on the highest Cry3Bbl.11098 expression level measured in pollen and leaf tissue of MON 863 corn plants. The maximum environmental concentration for soil dwelling organisms is predicted to be 13.3 mg/kg based on the assumption that corn plants are tilled into the top 6" of soil at the time of maximum leaf expression for Cry3Bbl .11098 (*i.e.* 93 ptg/g). (2) The DT_{50} and DT_{90} estimates for Cry3Bb1 protein in soil were found to range from 0.9 to 2.3 days and 7.4 to 50 days respectively. (2)	Terbufos hydrolyzes at pH 5, 7, and 9 with a half-life of 2.2 weeks. Formaldehyde was the major degradate detected in this study. Aerobic soil metabolism study indicate that terbufos degrades in silt loam soil with a half-life of 26.7 days. The major degradates detected in this study included carbon dioxide, terbufos sulfoxide, and terbufos sulfone. Terbufos residues have a half-life of less than 40 days in field plots of loam soil treated with a 15 percent granular formulation at an application rate of 1 lb ai/A. The sampling protocol was inadequate to accurately assess the dissipation of terbufos residues in field soil and a new study is required. The available data reviewed by the Agency are not sufficient to fulfill data requirements nor to assess the environmental fate of terbufos. EPA is concerned about the potential for the two degradates, terbufos sulfoxide and sulfone, to leach to groundwater, and the potential for parent terbufos and the sulfoxide and sulfone degradates to runoff to surface water. Terbufos parent degrades rapidly to the sulfoxide and sulfone metabolites, and is considered moderately mobile. Terbufos sulfoxide and sulfone are more mobile and persistent than parent terbufos. The acute DWLOCs calculated for the general U.S. population is 8.1 Fg/L.	Tefluthrin is immobile in soil and, therefore, will not leach into ground water. Additionally, due to the insolubility and lipophilic nature of tefluthrin, any residues in surface water will rapidly and tightly bind to soil particles and remain with sediment, therefore not contributing to potential Tefluthrin is immobile in soil and, therefore, will not leach into ground water. Additionally, due to the insolubility and lipophilic nature of tefluthrin, any residues in surface water will rapidly and tightly bind to soil particles and remain with sediment, therefore not contributing to potential dietary exposure from drinking water. Plant metabolism studies indicate that tefluthrin per se is not translocated to plants but is degraded in soil to two principal metabolites that are capable of being taken up by plants. EPA has decided that Metabolite VI need not be regulated. Based on tefluthrin not being registered for residentia1 non-food sites, EPA concludes that the aggregate short- and intermediate-term risks do not exceed levels of concern (MOE 1ess than 100), and that there is reasonable certainty that no harm will result from aggregate exposure to tefluthrin residues. (8)

			The chronic DWLOCs calculated for the general U.S. population is 1.7 Fg/L. Maximum acute and chronic estimated environmental concentrations (EECs) for parent terbufos plus the sulfoxide and sulfone degradates exceed the acute and chronic DWLOCs, respectively, in all cases. (5)	
Avian toxicity	Feeding Cry34/35Ab1 grain from event 15344 to chicken broilers resulted in no adverse effects on mortality, weight gain, feed efficiency and carcass yields. (1) LC_{50} ICP >25.1 ng active ingredient/mg diet LC_{50} Cry34Ab1 >23 ng active ingredient/mg diet LC_{50} Cry35Ab1 >2.1 ng active ingredient/mg diet (1)	Feeding of Cry3Bb-containing grain to Bobwhite quail at 10 and 35% of their diets, respectively, resulted in no adverse effects on growth or survival (2). The dietary LC_{50} value for Cry3Bb1 corn grain to juvenile Northern Bobwhite was greater than 70,000 ppm (10% of the diet) in eight day observation. (3)	Seven incidents to nontarget terrestrial organisms have been reported. Up to three of the incidents had some indication of misuse or misapplication. All the mortalities involved bird species (mostly raptors), with the exception of one incident involving red wolves in North Carolina, which is believed to be the result of an intentional poisoning. Calculated RQs for birds and mammals significantly exceed EPA=s risk concern for both granular formulations. (5) Dietary Avian Toxicity: 143 and 157 ppm (from two bobwhite studies). Avian Reproduction: Terbufos was not considered to produce avian reproductive effects based on results of a bobwhite quail study and a mallard duck study. (6)	Low toxicity to birds (9).
Fish toxicity	Feeding Cry34Ab1 microbially-produced protein and Cry35Ab1 microbially produced insect control protein to rainbow trout resulted in no adverse effects. (1) LC_{50} ICP >100 mg active ingredient/kg diet	Feeding of Cry3Bb-containing grain to channel catfish at 10 and 35% of their diets, respectively, resulted in no adverse effects on growth or survival. (2)	EPA has concerns about risk to nontarget aquatic organisms from parent terbufos and the terbufos sulfoxide and sulfone degradates based on widespread fish kill incidents involving terbufos use on corn with all application methods. These concerns are further supported by standard LOC criteria, which indicate risk concerns to	Highly toxic to fish (9)

	LC_{50} Cry34Ab1 >25 mg active ingredient/kg diet LC_{50} Cry35Ab1 >75 mg active ingredient/kg diet (1)		aquatic fish and invertebrates associated with both the clay-based (15% active ingredient) and polymer-based (20% active ingredient) granular formulations using banded applications.(5) Terbufos ranks fourth in pesticide-induced fish kills reported to the Agency, and is the leading cause of fish kills from use on corn. **Freshwater Fish Acute Toxicity**: Ranges from 0.77 to 20.00 ppb. - Freshwater Invertebrate Acute Toxicity: 0.31 ppb for Daphnia magna. - Marine/Estuarine Fish Acute Toxicity: Data gap. Marine/Estuarine Invertebrate Toxicity: Data gap. Mollusk toxicity: Data gap (5)	
Nontarget and beneficial organisms	Cry34Ab1 and Cry35Ab1 microbially produced protein were fed to non-target insects and resulted in no adverse effects. (1) **Green lacewing:** LC_{50} ICP >280 µg active ingredient/mL diet; LC_{50} Cry34Ab1 >160 µg active ingredient/mL diet; LC_{50} Cry35Ab1 >120 µg active ingredient/mL diet (1) **Parasitic Hymenoptera:** LC_{50} ICP >280 µg active ingredient/mL diet; LC_{50} Cry34Ab1 >160 µg active ingredient/mL diet; LC_{50} Cry35Ab1 >120 µg active ingredient/mL diet (1) **Lady beetle:** LC_{50} ICP >280 µg active ingredient/mL diet; LC_{50} Cry34Ab1 >160 µg active ingredient/mL diet;	Various "nontarget" organisms were exposed to high doses of leaf tissue, grain or pollen expressing Cry3Bb or to an artificial diet containing the purified protein for varying periods of time. They demonstrate that Cry3Bbl.11098 protein in MON 863 poses no significant risk for harm to nontarget organism populations. (2) **Green lacewing:** LC_{50} >8,000 ppm Cry3Bb1 protein (3) **Parasitic Hymenoptera:** LC_{50} >400 ppm Cry3Bb1 protein (3) **Lady beetle:** LC_{50} for adult *H. convergens* is >8,000 µg Bt protein/mL diet**;** LC_{50} for *C. maculate* >93 µg/g fresh pollen weight (3)	Terrestrial Field Study (Level 1): both soil-incorporated (2 lb ai/A) and nonsoil-incorporated (1 lb/A) resulted in nontarget mortalities, with the latter application much more severe in its effects (5,6)	Data not found.

	LC_{50} Cry35Ab1 >120 µg active ingredient/mL diet (1) ***Daphnia magna***: EC_{50} ICP >100 mg active ingredient/mL diet; LC_{50} Cry34Ab1 >57 mg active ingredient/mL diet; LC_{50} Cry35Ab1 >43 mg active ingredient/mL diet (1)	***Daphnia magna*****:** LC_{50} >120 mg pollen/L (3)		
Honeybee toxicity	Honey bees were fed Cry34/35Ab1 pollen from event TC5639 and Cry34Ab1 and Cry35Ab1 microbially produced protein. (1) LC_{50} pollen >2 mg/larvae (0.056 µg Cry34/35Ab1 ICP/larvae) LC_{50} ICP >20 µg active ingredient/larvae LC_{50} Cry34Ab1 >3.2 µg active ingredient/larvae LC_{50} Cry35Ab1 >2.4 µg active ingredient/larvae (1)	**Adult Honey Bee:** Cry3Bbl.11231 in an artificial diet LC_{50} >360 ug/ml in diet NOEC > 3X predicted maximum Cry3Bbl concentration in pollen **Larval Honey Bee :** Cry3Bbl.11231 in water NOEC 2 1790 ug/ml in diet LC_{50} > 10X predicted maximum Cry3Bb I concentration in pollen. (2, 3)	Not described in available studies.	High toxicity to bees (10)
Mammalian toxicity	Cry34Ab1 and Cry35Ab1 microbially produced protein were fed to mice and no acute oral toxicity or adverse effects in terms of body weight, detailed clinical observations and gross-pathological lesions were observed. (1) LD_{50} >2700 mg Cry34Ab1/kg LD_{50} >1850 mg Cry35Ab1/kg	**Acute toxicity**: Bt is practically non-toxic to humans and animals. Humans exposed orally to 1000 mg/day of Bt showed no effects.. No oral toxicity was found in rats, or mice fed protein crystals from Bt var. israelensis. The LD_{50} is greater than 5000 mg/kg for the Bt product Javelin in rats and greater than 13,000 mg/kg in rats exposed to the product Thuricide.	**Acute Oral**: Toxicity Category I (1.6 and 1.3 mg/kg for male and female rats, respectively). - **Acute Dermal**: Toxicity Category I (0.81 and 0.93 mg/kg for male and female rabbits, respectively). - **Acute Inhalation**: Toxicity Category I (< 0.2 mg/L). - **Delayed Neurotoxicity**: No evidence of acute delayed neurotoxicity at the 40	**Acute toxicity** studies with the technical grade of the active ingredient tefluthrin: oral LD50 in the rat is 21.8 mg/kg for males and 34.6 mg/kg for females; dermal LD50 in the rat is 316 mg/kg in males and 177 mg/kg in females; acute inhalation LC50 in the rat is 0.037 mg/l and 0.049 mg/l in male and female rats, respectively; primary dermal irritation study in the rabbit

	LD_{50} >2000 mg Cry34/35Ab1/kg (1)	Single oral dosages of up to 10,000 mg/kg did not produce toxicity in mice, rats, or dogs. The dermal LD_{50} for a formulated Bt product in rabbits is 6280 mg/kg. A single dermal application of 7200 mg/kg of Bt was not toxic to rabbits. Bt is an eye irritant; 100 grams of formulated product applied in congestion of the iris as well as redness and swelling. **Chronic toxicity**: No complaints were made by 8 men after they were exposed for 7 months. **Dietary administration** of Bt for 13 weeks to rats at dosages of 8400 mg/kg/day did not produce toxic effects. Some reversible abnormal redness of the skin was observed when 1 mg/kg/day of formulated Bt product was put on scratched skin for 21 days. No general, systemic poisoning was observed **Reproductive effects**: No indication. **Teratogenic effects**: No evidence. **Mutagenic effects**: There is no evidence of mutagenicity in mammalian species. **Carcinogenic effects**: It is unlikely that Bt is carcinogenic. (4).	mg/kg dosage level tested in hens. - **Subchronic Feeding**: The NOEL for both systemic effects and cholinesterase inhibition in a rat subchronic study is 0.25 ppm. - **Subchronic Dermal**: The NOEL for systemic effects in a 30-day rabbit study is 0.020 mg/kg. - **Mutagenicity**: Terbufos did not exhibit mutagenic potential in the Ames assay, the in vivo cytogenetic assay, and the dominant lethal test. - **Teratogenicity**: The NOEL for developmental toxicity in a rat teratology study is 0.1 mg/kg/day. - **Reproduction**: The NOEL for reproductive effects in a three-generation rat reproduction study is 0.25 ppm. - **Oncogenicity**: None (5,6)	showed slight irritation; and the acute delayed neurotoxicity study did not show acute delayed neurotoxicity. In an oral toxicity study, the NOEL for female rats is 100 ppm (equivalent to approximately 5 mg/kg/day). The NOEL for skin effects in rats is 1.0 mg/kg). The NOEL for neurological effects (the observed postural effects) may be between 0.025 and 0.1 mg/kg. **Carcinogenicity**: There was no evidence of carcinogenic potential. **Mutagenicity**: There is no mutagenicity concern. **Metabolism**: In both rats and dogs, when given either 1 or 10 mg/kg, most of the radioactivity was found in the feces unchanged and most urinary metabolites were conjugated. In rats, the halflife in the liver is 4.8 days, in the fat is 13.3 days and in the blood is 10.6 days. In a study with rat fat, half of the radioactive residues could be attributed to the parent and the remaining residues consisted of a mixture of fatty acid esters of hydroxylated parent metabolites. **Neurotoxicity**: No acceptable mammalian neurotoxicity studies are available.(8)
Nontarget soil organism effects	Cry34Ab1 and Cry35Ab1 microbially produced protein were fed to Collembola and earthworms and no adverse effects were observed. (1) **Collembola:** LC_{50} ICP >12.7 mg active ingredient/kg diet; LC_{50} Cry34Ab1 >3.2 mg active ingredient/kg diet; LC_{50}	The maximum environmental concentration for soil dwelling organisms is predicted to be 13.3 mg/kg based on the assumption that corn plants are tilled into the top 6" of soil at the time of maximum leaf expression for Cry3Bbl .11098 *(i.e.* 93 ptg/g). The measured NOECs from these tests	Not described by present reports.	Not found in these reports

	Cry35Ab1 >9.5 mg active ingredient/kg diet (1) **Earthworms:** LC_{50} ICP >25.4 mg active ingredient/kg diet; LC_{50} Cry34Ab1 >6.4 mg active ingredient/kg diet; LC_{50} Cry35Ab1 >19.0 mg active ingredient/kg diet (1)	exceed the maximum predicted environmental concentration by 3- to 140-fold, demonstrating an adequate margin of safety for these organisms (2). **Collembola:** LC_{50} >872.5µg protein (50% corn leaf tissue in the diet)m (3) **Earthworms:** LC_{50} 570 mg Cry3Bb1 protein/kg dry soil (3)		
Toxicity	Not assigned	Class III	Classified by EPA as Toxicity Category I	Toxicity class I for dermal, oral, inhalation exposures, and Class IV for skin irritation.
EDF=s Integrated Environmental Rankings - Combined human & ecological scores (7)	Not ranked	Not ranked	85-100% where 0 is the lowest and 100 is the highest hazard rating (7).	Data lacking; not ranked by any system in Scorecard.

Source of information:

1. Petition for Determination of Nonregulated Status for *Bt* Cry34/35Ab1 Insect-Resistant, Glufosinate-Tolerant Corn: Corn Line 59122-7.
2. Petition for Determination of Nonregulated Status for the Regulated Article: Corn Rootworm Protected Corn Event MON 853 (2000), Monsanto Company, St. Louis, MO.
3. EPA Biopesticide Registration Action Document (BRAD) http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm
4. Extoxnet: Extension Toxicology Network, Pesticide Information Profiles http://ace.ace.orst.edu/info/extoxnet/pips/bacillus.htm
5. Overview of Revised Terbufos Risk Assessment, Office of Pesticide Programs-- US EPA http://www.epa.gov/pesticides/op/terbufos/terbufosview.htm
6. EPA Pesticide Fact Sheet http://pmep.cce.cornell.edu/profiles/insect-mite/propetamphos-zetacyperm/terbufos/ insect-prof-terbufos.html
7. Environmental Defense Fund Scorecard. http://www.scorecard.org/chemical-profiles/
8. Tefluthrin; Pesticide Tolerance ENVIRONMENTAL PROTECTION AGENCY (40 CFR Part 180) [Federal Register: November 26, 1997 (Volume 62, Number 228)

http://www.epa.gov/fedrgstr/EPA-PEST/1997/November/Day-26/p30946.htm

9. Farm Chemicals Handbook, p. C374.

10. Ohio State University, Insect Pests of Field Crops Bulletin 545 AToxicity of Pesticides@ http://www.ag.ohio-state.edu/b45/b45_48.html

Appendix D. Data submitted with the petition in support of nonregulated status for *Bt cry3Bb1* corn line MON 88017

Molecular Genetic Characterization and Stability

Southern blot of event MON 88017 for analysis of insert and number, Fig. V-3, pg. 55.

Southern blot of event MON 88017 for analysis of intactness of the *cp4 epsps* cassette probed with P-ract + Ract1 intron probe, Fig. V-4, pg. 57.
Southern blot of event MON 88017 for analysis of intactness of the *cp4 epsps* cassette probed with CTP2 + *cp4 epsps* probe, Fig. V-5, pg. 59.

Southern blot of event MON 88017 for analysis of intactness of the *cp4 epsps* cassette probed with NOS 3' probe, Fig. V-6, pg. 60.

Southern blot of event MON 853 for analysis of intactness of the *cry3Bb1* cassette probed with P-e35S probe, Fig. V-7, pg. 63.

Southern blot of event MON 853 for analysis of intactness of the *cry3Bb1* cassette probed with wt CAB leader + Ract1 intron probe, Fig. V-8, pg. 64.

Southern blot of event MON 853 for analysis of intactness of the *cry3Bb1* cassette probed with *cry3Bb1* probe, Fig. V-9, pg. 65.

Southern blot of event MON 853 for analysis of intactness of the *cry3Bb1* cassette probed with tahsp17 3' probe, Fig. V-10, pg. 66.

Southern blot of event MON 853 confirming absence of backbone sequences, Fig. V-11, pg. 68.

Breeding tree across multiple generations MON 88017, Fig. V-12, pg. 70.

Stability of integrated DNA across multiple generations of MON 88017, Fig. V-13, pg. 71.

Overlapping PCR analysis of insert in MON 88017 confirming insert organization (Part 1), Fig. V-14, pg. 73.

Overlapping PCR analysis of insert in MON 88017 confirming insert organization (Part 2), Fig. V-14, pg. 73.

Chi square analysis of expected and observed frequencies for the introduced trait indicating stability and predictable Mendelian inheritance in MON 88017, Table V-1, pg. 76.

Phenotypic Characterization and Evidence Supporting Absence of Unintended Effects

Measurements of Cry3Bb1 and CP4 EPSPS protein levels in various tissues collected from multiple filed sites Table VI-1, pg. 85.

Measurements of CP4 EPSP protein levels in over-season tissues of MON 88017, Table VI-2, pg. 86.

Measurements of Cry3Bb1 protein levels in over-season tissues of MON 88017, Table VI-3, pg. 87

Limits of detection and quantitation for ELISA analysis of CP4 EPSPS and Cry3Bb1 proteins, Table VI-4, pg. 88.

Measurements of phenotypic and ecological characteristics for MON 88017, Table VII-1, pg. 90.

Measurement of phenotypic comparison for MON 88017 and control plants in 2001 field trials, Table VII-3, pg. 97.

Evaluation of insect, disease and abiotic stress susceptibility for 2001 field trials, Table VII-4, pg. 98.

Measure of phenotypic comparison for MON 88017 and control plants in 2002 field trials, Table VII-5, pg. 101.

Evaluation of insect, disease and abiotic stress susceptibility for 2002 field trials, Table VII-6, pg. 102.

Measurement of MON 88017 and control pollen, Table VII-7, pg. 104.

Compositional analysis of grain collected from corn event MON 88017, and non-transgenic control corn varieties, Table VII-8, pg. 109-110.

Dormancy and germination evaluation of corn rootworm protected corn for an ecological assessment of weediness. Appendix E, pg. 225-230.

Morphological and viability assessment of event MON 88017 corn pollen, Section VII.A.7, Figures VII-1 and VII-2, pg. 103-107.

Data on environmental consequences of introduction MON 863

Susceptibility of various insect pests to Cry3Bb1 extracted from recombinant Bt. strain 11231 and 11098 (MON 863) in laboratory dietary bioassays, Table VIII-1, pg. 117.

Major insect pests of U.S. corn, Table VIII-5, pg. 130.

Troublesome weeds in U.S. corn, Table VIII-6, pg. 131.

Percent of corn acres in the U.S. Corn Belt treated in 2003 with herbicides, Table VIII-7, pg. 133.

Appendix E. Changes in Herbicide Use in Corn Since 1999-2003

Herbicide	1999[b]		2003[a]		% Change[d]
	% Area Treated	Total Applied[c]	% Area Treated	Total Applied[c]	
Acetochlor	27	31,824	26	36,067	13.3
Atrazine	70	54,780	68	55,642	1.5
Glufosinate-Ammonium	*	*	3	833	-
Glyphosate	9	4,162	19	11,913	186.2
Mesotrione	*	*	13	976	-
Metolachlor	29	29,554	6	6,384	-78.4
Nicosulfuron	15	150	11	166	10.6
States Surveyed	CO, IL, IN, IA, KS, KY, MI, MN, MO, NE, NC, OH, SD, TX, WI		CO, IL, IN, IA, KS, KY, MI, MN, MO, NE, NY, NC, ND, OH, PA, SD, TX, WI		

[a] USDA-NASS. 2004. Agricultural Chemical Usage 2003 Field Crops Summary.
[b] USDA-NASS. 2000. Agricultural Chemical Usage 1999 Field Crops Summary
[c] 1000 lbs/acre
[d] Percent change in total lbs applied
[*] No data available for that growing season