BIOPESTICIDES REGISTRATION ACTION DOCUMENT

Bacillus thuringiensis Cry3Bb1 Protein and the Genetic Material Necessary for Its Production (Vector PV-ZMIR13L) in MON 863 Corn (OECD Unique Identifier: MON-ØØ863-5)

PC Code: 006484

Bacillus thuringiensis Cry3Bb1 Protein and the Genetic Material Necessary for Its Production (Vector PV-ZMIR39) in MON 88017 Corn (OECD Unique Identifier: MON-88Ø17-3)

PC Code: 006498

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division

Table of Contents

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I. OVERVIEW

A. Background

On February 24, 2003, the Environmental Protection Agency (EPA) issued a time-limited, conditional registration to Monsanto Company ("Monsanto") for Corn Event MON 863 (EPA Reg. No. 524-528), a plant-incorporated protectant expressing the active ingredient, *Bacillus thuringiensis* (*Bt*) Cry3Bb1 protein and the genetic material necessary for its production (vector PV-ZMIR13L) in MON 863 corn (Organization for Economic Cooperation and Development (OECD) Unique Identifier: MON-ØØ863-5). Corn Event MON 863, at the time of 2003 registration, expressed the first plant-incorporated protectant (PIP) active ingredient to offer protection against corn rootworm, and expectations were that adaptation of this new technology would result in reduction of conventional insecticide use (e.g., organophosphates, carbamates, and synthetic pyrethroids) by growers attempting to control the highly destructive corn rootworm and maintain their crop yields. Prior to registration and after extensive review of copious amounts of data/information submitted by the applicant, the Agency determined that the use of this pesticide was in the public interest and that it would not cause any unreasonable adverse effects on the environment during the period of time-limited (less than a year), conditional registration. The time limitation on this particular registration was extended twice by the Agency: first from May 1, 2004 to July 31, 2006 and then from July 31, 2006 to September 30, 2010.

Subsequent to registration of the single-trait product in 2003, the Agency registered another single-trait Cry3Bb1 product (MON 88017; EPA Reg. No. 524-551) and several stacked and/or pyramided plant-incorporated protectants (PIPs), expressing Cry3Bb1 along with other proteins, for either commercial or limited breeding purposes. A complete list of the currently registered products expressing Cry3Bb1—including their respective registration numbers, product names, registrants, initial dates of registration, proteins (or active ingredients) expressed, and any limitations/special notes—can be found in Appendix A. In conjunction with the 2010 evaluation (explained in the paragraphs that follow), the Agency attempted to better detail and describe product characterization, human health, environmental effects, and insect resistance management (IRM) data that were submitted to support the registration of MON 88017 and the following stacked products expressing Cry3Bb1:

(1) MON 863 x MON 810 (EPA Reg. No. 524-545)
(2) MON 88017 x MON 810 (EPA Reg. No. 524-552)

When the first products containing the two Cry3Bb1 protein variants were registered by the Agency, Monsanto was issued time-limited, conditional registrations under section 3(c)(7)(C) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Along with several requirements for further product characterization, environmental effects, and IRM data, the registration notices also clearly established absolute expiration dates. Although the registrations—specifically Corn Event MON 863, MON 863 x MON 810, MON 88017, and MON 88017 x MON 810—began with varying absolute expiration dates, they were set to expire on September 30, 2010. Monsanto formally requested that the Agency amend their MON 88017 and MON 88017 x MON 810 registrations to extend the current expiration date. Conversely, for the Corn Event MON 863 and MON 863 x MON 810 products,

Monsanto did not seek an extension to the expiration date; therefore, these registrations expired on their own terms, and the Agency issued a cancellation order, outlining provisions for existing stocks (75 FR 52329, August 25, 2010).

On October 1, 2009, EPA announced a policy to provide a more meaningful opportunity for the public to participate on major registration decisions before they occur. According to this policy, EPA intends to provide a public comment period prior to making a registration decision for, at minimum, the following types of applications: new active ingredients; first food uses; first outdoor uses; first residential uses; and other actions for which the Agency anticipates that there will be significant public interest.

Consistent with the policy of making registration actions more transparent, the amendments to the expiring Cry3Bb1 corn products were subject to a 30-day comment period because the Agency believed, given past experiences with PIPs in general, these actions would be of significant interest to the public. During this comment period, several comments were received from the following stakeholders: Mycogen Seeds c/o Dow AgroSciences LLC; Pioneer Hi-Bred International, Incorporated; Monsanto Company; National Corn Growers Association; Agricultural Biotechnology Stewardship Technical Committee; Center for Science in the Public Interest; and Association of American Seed Control Officials. After reviewing and considering all of the public comments received, the Agency still maintains that, based on all data submitted in support of the Cry3Bb1 corn registrations (both for initial registrations and as responses to conditions of registration), it is in the best interest of the public and the environment to amend the currently existing Cry3Bb1 registrations by extending the current expiration dates in accordance with the scheme explained in section III(E) of this Biopesticides Registration Action Document (BRAD). The basis for this decision can be found in both the risk assessment for the Cry3Bb1 corn products, which is characterized throughout this BRAD, and the Agency's response to comments document.

All data and findings for the Cry3Bb1 corn products are presented within the standard BRAD configuration for PIPs (i.e., information is placed into separate and distinct chapters according to scientific discipline or regulatory focus); this should be the most familiar format to outside stakeholders interested in reading further about these actions. In addition to the Cry3Bb1 corn products, there are other *Bt* corn PIPs, expressing different proteins effective in controlling corn borers or corn rootworm, that were due to expire in 2010, and for which the associated registrants formally requested an extension to expiration dates. Therefore, within the same docket (EPA-HQ-OPP-2010-0607) as this document, the following information[a] is also available for public examination:

- Cry1F and Cry1Ab BRAD (Draft - August 2010; Final - September 2010)
- Cry3Bb1 BRAD (Draft - July 2010; Final - September 2010)
- mCry3A BRAD (Draft - July 2010; Final - September 2010)

[a] Each of the Biopesticides Registration Action Documents in this action are modified from previous versions to account for data/information submitted to fulfill terms and conditions of registration (see draft and final versions) and to respond, in part, to comments received on the information presented in Docket Number EPA-HQ-OPP-2010-0607 (see final versions only). All documents presented in the list can be retrieved from the following website: *http://www.regulations.gov*.

- Cry1A.105 and Cry2Ab2 BRAD (Draft - August 2010; Final - September 2010)
- Optimum® AcreMax™ *B.t.* Seed Blends BRAD (Draft - August 2010; Final - September 2010)
- Current Registration Terms and Conditions for *Bt* Corn Registrations Set to Expire in 2010
- Proposed Registration Terms and Conditions for *Bt* Corn Registrations Set to Expire in 2010
- Registration Terms and Conditions Established with the Finalized Amendments
- BPPD mCry3A, Cry3Bb1, and Cry34/35Ab1 Corn Rootworm Monitoring Reviews (June 2010)
- Public Comments on EPA Docket Number EPA-HQ-OPP-2010-0607
- EPA's Response to Comments

EPA made the decision to amend the registrations of eighteen (18) expiring *Bt* corn PIP registrations to extend the expiration dates. We conducted comprehensive assessments of each of these registrations, considering all toxicity and environmental effects data, data from insect resistance monitoring, and insect resistance refuge compliance reports, received and obtained since the last comprehensive evaluation of these products in 2001. Based upon our comprehensive assessment, we reached significant conclusions regarding the positive environmental impact of *Bt* corn PIPs, and we took several actions to strengthen the insect resistance management requirements to ensure continued success in the prevention of the evolution of resistance in target pests.

Since the commercialization of *Bt* crops, there have been a significant number of published field studies that, combined with the post-registration field studies required to be submitted to the Agency, have demonstrated that non-target invertebrates are generally more abundant in *Bt* cotton and *Bt* corn fields than in non-transgenic fields managed with chemical insecticides. Thus, these published and registrant-produced studies demonstrate that, not only are the *Bt* crops not causing any unreasonable adverse effects in the environment, but, arthropod prevalence and diversity is greater in *Bt* crop fields.

To strengthen insect resistance management of these corn PIPs and to address reports that compliance with the mandated refuge requirements has been decreasing, EPA is requiring enhanced compliance assurance programs (CAPs), and a phased requirement for seed bag labeling that clearly shows the refuge requirements. Also, given the increasing variety of PIP products and combinations, and the differing risk of resistance evolution that the various products represent, we are granting registrations for the corn PIP products for different time frames, based on assessments of their likelihood of forestalling the evolution of insect resistance. We are registering differing categories of products for differing time periods to reflect the assessed level of risk of resistance posed by the various corn PIP products. The scheme that we are following includes registration periods generally of five, eight, and twelve years; with the possibility of a fifteen-year registration period for products that are demonstrated to meet specified criteria. We retain, however, the discretion to register products for time periods differing from these defaults where circumstances warrant.

B. Use Profile

1. Corn Event MON 863

Pesticide Name: *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector PV-ZMIR13L) in MON 863 corn (OECD Unique Identifier: MON-ØØ863-5)

Trade and Other Names: Corn Event MON 863 and YieldGard® Rootworm

OPP Chemical Code: 006484

Basic Manufacturer: Monsanto Company
800 North Lindbergh Boulevard
St. Louis, Missouri 63167

Type of Pesticide: Plant-Incorporated Protectant

Use: Field Corn

Target Pests: western corn rootworm (*Diabrotica virgifera virgifera*), northern corn rootworm (*Diabrotica barberi*), and Mexican corn rootworm (*Diabrotica virgifera zeae*)

Products Expressing This Pesticide: See complete list in Appendix A.

2. MON 88017

Pesticide Name: *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector PV-ZMIR39) in MON 88017 corn (OECD Unique Identifier: MON-88Ø17-3)

Trade and Other Names: MON 88017

OPP Chemical Code: 006498

Basic Manufacturer: Monsanto Company
800 North Lindbergh Boulevard
St. Louis, Missouri 63167

Type of Pesticide: Plant-Incorporated Protectant

Use: Field and Sweet Corn

Target Pests: western corn rootworm (*Diabrotica virgifera virgifera*), northern corn rootworm (*Diabrotica barberi*), and Mexican corn rootworm (*Diabrotica virgifera zeae*)

Products Expressing This Pesticide: See complete list in Appendix A.

C. Regulatory History

Date	Action Type	Description
October 10, 1997[1]	Federal Register Publication (Notice of Filing)	Notice of Filing summarizing information submitted and cited by Monsanto Company in support of a request for establishment of an exemption from the requirement of a tolerance for residues of the plant pesticides consisting of *Bacillus thuringiensis* Cry1, Cry2, and Cry3 classes of proteins and the genetic material necessary for the production of these proteins in or on all raw agricultural commodities. (62 Federal Register (FR) 52998)
December 8, 1999[2]	Federal Register Publication (Notice of Receipt)	Notice announcing receipt of applications 524-EUP-ON, 524-EUP-OE, and 524-EUP-OG from Monsanto Company requesting experimental use permits for the following: (1) *Bacillus thuringiensis* Cry3Bb protein and the genetic material necessary for its production (vector ZMIR14L) in corn (2) *Bacillus thuringiensis* Cry3Bb protein and the genetic material necessary for its production (vector ZMIR12L) in corn (3) *Bacillus thuringiensis* Cry3Bb protein and the genetic material necessary for its production (vector ZMIR13L) in corn (64 FR 68681)
December 28, 2000[2]	Federal Register Publication (Notice of Receipt)	Notice announcing receipt of an application from Monsanto Company requesting amendment/extension to its experimental use permit, 524-EUP-93 (involving testing of *Bacillus thuringiensis* Cry3Bb protein and the genetic material necessary for its production (vector ZMIR13L) in corn only). (65 FR 82352)
January 17, 2001[2]	Federal Register Publication (Notice of Issuance)	Notice announcing issuance of experimental use permits to Monsanto Company (524-EUP-90, 524-EUP-92, and 524-EUP-93). The permits allowed for use of (1) *Bacillus thuringiensis* Cry3Bb protein and the genetic material necessary for its production (vector ZMIR14L) in corn, (2) *Bacillus thuringiensis* Cry3Bb protein and the genetic material necessary for its production (vector ZMIR12L) in corn, and (3) *Bacillus thuringiensis* Cry3Bb protein and the genetic material necessary for its production (vector ZMIR13L) in corn on 1,343 acres of corn (524-EUP-90), on 416 acres of corn (524-EUP-92), and on 1,092 acres of corn (524-EUP-93). All of these experimental use permits were effective from April 6, 2000 to April 31, 2001. The permits were issued with the limitation that all treated crops were to be genetically contained and destroyed or used for research purposes only. (66 FR 4020)

Date	Action Type	Description
March 19, 2001[2]	Federal Register Publication (Notice of Receipt)	Notice announcing receipt of the following application for a product containing a new active ingredient: (1) File Symbol: 524-LEI. Applicant: Monsanto Company, 700 Chesterfield Parkway North, St. Louis, MO 63198. Product Name: Event MON 863: Corn Rootworm Protected Corn (ZMIR13L). Type of Product: Plant-pesticide. Active Ingredient: *Bacillus thuringiensis* Cry3Bb protein and the genetic material (vector ZMIR13L) necessary for its production in corn. *For 1 year, contained, 22,875 acre pre-commercial inbred seed propagation and hybrid seed production registration. Plantings are proposed for the states of California, Hawaii, Illinois, Iowa, Indiana, Kansas, Michigan, Nebraska, South Dakota, Texas, and Wisconsin. (66 FR 15435)
May 11, 2001[1]	Federal Register Publication (Final Rule)	The following temporary exemption from the requirement of a tolerance was established under 40 Code of Federal Regulations (CFR) § 180.1214: "*Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production in corn are exempt from the requirement of a tolerance when used as plant-pesticides in the food and feed commodities of field corn, sweet corn, and popcorn. Genetic material necessary for its production means the genetic material which comprise genetic material encoding the Cry3Bb1 protein and its regulatory regions. Regulatory regions are the genetic material, such as promoters, terminators, and enhancers, that control the expression of the genetic material encoding the Cry3Bb1 protein. This exemption from the requirement of a tolerance will expire on May 1, 2004." (66 FR 24061)
July 27, 2001[2]	Federal Register Publication (Notice of Issuance)	Notice announcing extension/amendment of an experimental use permit previously approved for Monsanto Company (524-EUP-93): For use of 7.4 pounds of *Bacillus thuringiensis* Cry3Bb protein and the genetic material necessary for its production (vector ZMIR13L) in corn on 4,000 acres of field corn; Effective from April 27, 2001 to April 2002. (66 FR 39163)

Date	Action Type	Description
September 11, 2001[2]	Federal Register Publication (Notice of Receipt)	Notice announcing receipt of an application from Monsanto Company requesting amendment to its experimental use permit, 524-EUP-93 (involving testing of *Bacillus thuringiensis* Cry3Bb protein and the genetic material necessary for its production (vector ZMIR13L) in corn only). Amendment requested to allow livestock feeding studies and lift the crop destruct provisions, given the temporary tolerance exemption established on May 11, 2001 for *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production in corn. (66 FR 47218)
February 20, 2002[2]	Federal Register Publication (Notice of Receipt)	Notice announcing receipt of an application from Monsanto Company requesting amendment/extension to its experimental use permit, 524-EUP-93 (involving testing of *Bacillus thuringiensis* Cry3Bb protein and the genetic material necessary for its production (vector ZMIR13L) in corn only). (67 FR 7687)
March 13, 2002[2]	Federal Register Publication (Notice of Receipt)	Notice announcing receipt of the following application for a product containing a new active ingredient: (1) File Symbol: 524-LEI. Applicant: Monsanto Company, 700 Chesterfield Parkway North, St. Louis, MO 63198. Product Name: Event MON 863: Corn Rootworm Protected Corn (ZMIR13L). Type of Product: Plant-pesticide. Active Ingredient: *Bacillus thuringiensis* Cry3Bb protein and the genetic material (vector ZMIR13L) necessary for its production in corn. *Updated the March 19, 2001 Notice of Receipt in that Monsanto Company changed their application for registration from limited use (in certain states, for a certain amount of acreage, and for seed increase purposes only) to full commercial use. (67 FR 11333)
May 1, 2002[2]	Federal Register Publication (Notice of Extension of Comment Period)	Notice announcing extension of the comment period for the updated Notice of Receipt published in the Federal Register on March 13, 2002. Originally, the comment period ended on April 12, 2002, but the Agency made the decision to extend the comment period to May 31, 2002. (67 FR 21669)

Date	Action Type	Description
June 26, 2002[2]	Federal Register Publication (Notice of Issuance)	Notice announcing extension/amendment of an experimental use permit previously approved for Monsanto Company (524-EUP-93): Removed the crop destruct requirement set forth with the original issuance of this experimental use permit; For use of *Bacillus thuringiensis* Cry3Bb protein and the genetic material necessary for its production (vector ZMIR13L) in corn on 9,400 acres of field corn; Effective from April 10, 2002 to February 28, 2003. (67 FR 43115)
February 24, 2003[2]	Registration	The Agency issued a time-limited, conditional registration notice (under FIFRA section 3(c)(7)(C)) for Corn Event MON 863 (EPA Reg. No. 524-528). *Expiration Date: May 1, 2004
April 2, 2003[2]	Federal Register Publication (Notice of Receipt)	Notice announcing receipt of the following application for a product containing an active ingredient involving a changed use pattern: (1) File Symbol: 524-LUL. Applicant: Monsanto Company, 700 Chesterfield Parkway North, St. Louis, MO 63198. Product Name: YieldGard Plus Corn. Type of Product: Plant-incorporated protectant. Active Ingredients: *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR13L) in corn and *Bacillus thuringiensis* Cry1Ab protein and the genetic material necessary for its production in corn. (68 FR 16036)
April 2, 2003[3]	Federal Register Publication (Notice of Receipt)	Notice announcing receipt of application 524-EUP-OA from Monsanto Company requesting an experimental use permit for *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR39) in corn. (68 FR 16050)
April 23, 2003[2]	Federal Register Publication (Notice of Receipt)	Notice announcing receipt of an application from Monsanto Company requesting amendment/extension to its experimental use permit, 524-EUP-93 (involving testing of *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR13L) in corn only). (68 FR 19995)

Date	Action Type	Description
October 22, 2003[1]	Federal Register Publication (Notice of Filing)	Notice of Filing summarizing information submitted and cited by Monsanto Company in support of a request for establishment of an exemption from the requirement of a tolerance for the plant-incorporated protectant, *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production in corn in or on field corn, sweet corn, and popcorn. (68 FR 60371)
October 31, 2003[2]	Registration	The Agency issued a time-limited, conditional registration notice (under FIFRA section 3(c)(7)(B)) for YieldGard® Plus Corn (EPA Reg. No. 524-545) *Expiration Date: May 1, 2004
January 7, 2004[2]	Federal Register Publication (Notice of Issuance)	Notice announcing extension/amendment of an experimental use permit previously approved for Monsanto Company (524-EUP-93): For use of *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR13L) in corn MON 863 and *Bacillus* thuringiensis Cry1Ab delta-endotoxin and the genetic material necessary for its production (vector PV-ZMCT01) in corn MON 810 on 2,304 acres of field corn; Effective from June 20, 2003 to December 31, 2003. (69 FR 917)
January 7, 2004[3]	Federal Register Publication (Notice of Issuance)	Notice announcing issuance of an experimental use permit to Monsanto Company (524-EUP-96). The permit allowed for use of *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR39) in corn ZMIR39 and *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production (vector PV-ZMCT01) in corn MON 810 (ZMIR39, MON 810, and ZMIR39 x MON 810 hybrids) on 829.7 acres of corn. This experimental use permit was effective from July 2, 2003 to December 31, 2003. (69 FR 917)

Date	Action Type	Description
March 3, 2004[3]	Federal Register Publication (Notice of Receipt)	Notice announcing receipt of an application from Monsanto Company requesting amendment/extension to its experimental use permit, 524-EUP-96 (involving testing of *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR39) in corn ZMIR39 and *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production (vector PV-ZMCT01) in corn MON 810 (ZMIR39, MON 810, and ZMIR39 x MON 810 hybrids)). (69 FR 10040)
March 31, 2004[1]	Federal Register Publication (Final Rule)	The following permanent exemption from the requirement of a tolerance was established under 40 CFR § 180.1214: "*Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production in corn are exempt from the requirement of a tolerance when used as plant-incorporated protectants in the food and feed commodities of field corn, sweet corn, and popcorn. Genetic material necessary for its production means the genetic material which comprise genetic material encoding the Cry3Bb1 protein and its regulatory regions. Regulatory regions are the genetic material, such as promoters, terminators, and enhancers, that control the expression of the genetic material encoding the Cry3Bb1 protein." (69 FR 16809)
April 23, 2004[2]	Amendment	The Corn Event MON 863 (EPA Reg. No. 524-528) and YieldGard® Plus Corn (EPA Reg. No. 524-545) registrations were amended by the Agency to extend the expiration date set forth in the original registration notices. *New Expiration Date: July 31, 2006

Date	Action Type	Description
December 22, 2004[3]	Federal Register Publication (Notice of Receipt)	Notice announcing receipt of the following applications for products containing a new active ingredient: (1) File Symbol: 524-LLR. Applicant: Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO 63167. Product Name: MON 88017. Type of Product: Plant-incorporated protectant. Active Ingredient: *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR39) in MON 88017 corn. (2) File Symbol: 524-LLE. Applicant: Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO 63167. Product Name: MON 88017 x MON 810. Type of Product: Plant-incorporated protectant. Active Ingredients: *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR39) in MON 88017 corn and *Bacillus thuringiensis* Cry1Ab and the genetic material necessary for its production in corn. (69 FR 76716)
December 22, 2004[3]	Federal Register Publication (Notice of Issuance)	Notice announcing extension/amendment of an experimental use permit previously approved for Monsanto Company (524-EUP-96): For use of 2.8 pounds of *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR39) in corn ZMIR39 and *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production (vector PV-ZMCT01) in corn MON 810 (ZMIR39, MON 810, and ZMIR39 x MON 810 hybrids) on 2,530 acres of field corn; Effective from April 27, 2004 to February 28, 2005. (69 FR 76732)
January 12, 2005[3]	Federal Register Publication (Notice of Receipt)	Notice announcing receipt of an application from Monsanto Company requesting amendment/extension to its experimental use permit, 524-EUP-96 (involving testing of *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR39) in corn ZMIR39 and *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production (vector PV-ZMCT01) in corn MON 810 (ZMIR39, MON 810, and ZMIR39 x MON 810 hybrids)). (70 FR 2160)

Date	Action Type	Description
August 10, 2005[3]	Federal Register Publication (Notice of Issuance)	Notice announcing extension/amendment of an experimental use permit previously approved for Monsanto Company (524-EUP-96): For use of 3.63 pounds of the insecticides, *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR39) in corn ZMIR39 and *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production (vector PV-ZMCT01) in corn MON 810 (ZMIR39, MON 810, and ZMIR39 x MON 810 hybrids), on 4,683 acres of field corn; Effective from February 18, 2005 to March 1, 2006. (70 FR 46510)
October 19, 2005[2]	Federal Register Publication (Notice of Issuance)	Notice announcing conditional approval of two products: (1) Event MON 863: Corn Rootworm Protected Corn (ZMIR13L)(EPA Reg. No. 524-528) – Registered under FIFRA section 3(c)(7)(C) and containing *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR13L) in corn, an active ingredient not included in any previously registered product. (2) YieldGard Plus Corn (EPA Reg. No. 524-545) – Registered under FIFRA section 3(c)(7)(B) and containing *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR13L) in corn and *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production in corn. *Note: Over 900 comments were received by the Agency in response to the Notices of Receipt for EPA Reg. Nos. 524-528 and 524-545. The Agency's response to these comments can be found in Docket Number EPA-HQ-OPP-2004-0182 at *www.regulations.gov*. (70 FR 60826)
December 13, 2005[3]	Registration	The Agency issued time-limited, conditional registration notices (under FIFRA section 3(c)(7)(C)) for MON 88017 (EPA Reg. No. 524-551) and MON 88017 x MON 810 (EPA Reg. No. 524-552) *MON 88017 Expiration Date: September 30, 2010 *MON 88017 x MON Expiration Date: October 15, 2008

Date	Action Type	Description
July 27, 2006[2]	Amendment	Corn Event MON 863 (EPA Reg. No. 524-528) and YieldGard® Plus Corn (EPA Reg. No. 524-545) were amended by the Agency to extend the expiration date set forth in previous regulatory correspondence. *Corn Event MON 863 New Expiration Date: September 30, 2010 *YieldGard® Plus Corn New Expiration Date: October 15, 2008
April 25, 2007[1]	Federal Register Publication (Direct Final Rule)	The tolerance exemption for Cry3Bb1 was redesignated from 40 CFR § 180.1214 to 40 CFR § 174.518 and changed to the following: "Residues of *Bacillus thuringiensis* Cry3Bb1 protein in corn are exempt from the requirement of a tolerance when used as plant-incorporated protectants in the food and feed commodities of corn; corn, field; corn, sweet; and corn, pop." (72 FR 20431)
August 15, 2007[3]	Federal Register Publication (Notice of Issuance)	Notice announcing conditional approval of two products: (1) MON 88017 (EPA Reg. No. 524-551) – Registered under FIFRA section 3(c)(7)(C) and containing *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR39) in Event 88017 corn (OECD Unique Identifier: MON-88Ø17-3), an active ingredient not included in any previously registered product. (2) MON 88017 x MON 810 (EPA Reg. No. 524-552) – Registered under FIFRA section 3(c)(7)(C) and containing *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR39) in Event 88017 corn (OECD Unique Identifier: MON-88Ø17-3)(an active ingredient not included in any previously registered product) and *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production (vector PV-ZMCT01) in Event MON 810 corn (OECD Unique Identifier: MON-ØØ81Ø-6). (72 FR 45807)
October 10, 2008[1]	Amendment	YieldGard® Plus Corn (EPA Reg. No. 524-545) and MON 88017 x MON 810 (EPA Reg. No. 524-552) were amended by the Agency to extend the expiration date set forth in previous regulatory correspondence. *New Expiration Date: September 30, 2010

Date	Action Type	Description
June 2008–December 2009[3]	Registration	The Agency registered several combination PIP products expressing Cry3Bb1 and another protein(s). See Appendix A for the complete list.
August 25, 2010[2]	Federal Register Publication (Cancellation Order)	Monsanto did not request an extension to their Corn Event MON 863 (EPA Reg. No. 524-528) or MON 863 x MON 810 (EPA Reg. No. 524-545) registrations; therefore, these registrations expired on their own terms on September 30, 2010. The Agency considers the expiration of a conditional, time-limited registration to be a cancellation under FIFRA section 3. A cancellation order, effective September 30, 2010, and appropriate provisions for disposition of existing stocks published in the Federal Register on August 25, 2010. (75 FR 52329)
September 2010[3]	Amendment	The MON 88017 (EPA Reg. No. 524-551) and MON 88017 x MON 810 (EPA Reg. No. 524-552) registrations were amended by the Agency to extend the expiration date in accordance with the scheme explained in section III(E) of this Biopesticides Registration Action Document (BRAD). *New Expiration Date: September 30, 2015

[1] Applies to both Corn Event MON 863 and MON 88017
[2] Applies only to Corn Event MON 863
[3] Applies only to MON 88017

II. SCIENCE ASSESSMENT

The classifications that are found for each data submission are assigned by Environmental Protection Agency (EPA) science reviewers and are an indication of the usefulness of the information contained in the documents for risk assessment. A rating of "ACCEPTABLE" indicates the study is scientifically sound and is useful for risk assessment. A "SUPPLEMENTAL" rating indicates the data provide some information that can be useful for risk assessment. The studies may have certain aspects determined not to be scientifically acceptable ("SUPPLEMENTAL: UPGRADABLE"). If a study is rated as "SUPPLEMENTAL: UPGRADABLE," EPA always provides an indication of what is lacking or what can be provided to change the rating to "ACCEPTABLE." If there is simply a "SUPPLEMENTAL" rating, the reviewer will often state that the study is not required by the current 40 Code of Federal Regulations (CFR) Part 158. Both "ACCEPTABLE" and "SUPPLEMENTAL" studies may be used in the risk assessment process as appropriate. An "UNACCEPTABLE" rating indicates that the study is not useful for risk assessment and cannot be upgraded.

A. Product Characterization

1. General Background on Cry3Bb1-Expressing Products

Product characterization is critical to understanding the way in which the product was made and the unique characteristics that need to be assessed. The product characterization data provide information on the specific transformation systems used for each product, the actual deoxyribonucleic acid (DNA) inserted into the plant, the inheritance and stability of these traits in the plant, biochemical characteristics of the protein, and protein expression levels for various plant tissues.

a. Transformation System

Except for MON 88017, Cry3Bb1 plant-incorporated protectants were transformed into corn tissue via a method employing bombardment of particles coated with DNA encoding the intended insert. MON 88017, on the other hand, was produced by the *Agrobacterium*-mediated transformation of corn cells with plasmid vector PV-ZMIR39. Each plasmid description includes a reference to the strains of *Bacillus thuringiensis* (*Bt*) used as the source of the DNA sequence for the toxin protein. In addition, the sources for marker proteins, promoters, terminators, and enhancers, as well as the fragment size, orientation, and any modifications to the original DNA sequence to enhance expression in the plant, are given. All the other DNA sequences, improving or restricting expression of the introduced traits, are also described. Finally, the plasmid discussion includes a description of any modifications made to the DNA (e.g., codon modifications to improve eukaryotic expression).

b. Characterization of the DNA Inserted in the Plant

Inserted DNA is characterized with Southern blot data of the DNA in the plant genome. The analysis usually consists of DNA isolation from the transformed plant, digestion of this DNA with several different endonucleases, and hybridization of these restriction endonuclease fragments with labeled-

DNA that is complementary to the introduced traits. This analysis includes not only probes specific for the entire insert, but also probes recognizing just the coding regions of the traits or DNA elements outside the coding region. Polymerase chain reaction (PCR) assays—utilizing various specific and non-specific primers, genome walking, cosmid libraries, and DNA sequencing—have also been employed with sensitive Southern blotting techniques to more completely describe the inserted DNA and surrounding regions. The information available from these blots can indicate the presence of all the elements of the expected insert, as well as information about possible deletions and other errors associated with DNA introduction by transformation. Comparison of Southern blots of genomic DNA, digested using a range of restriction endonucleases, can also reveal the copy number of the genes introduced and suspected linkage of the traits. Alternatively, the intensity of the radioactive label from binding the probe DNA can also estimate the number of insert copies incorporated in the plant genome.

c. Inheritance and Stability after Transformation

The data generated for this endpoint examine progeny from crosses between selected elite lines with the transformed *Bt*-expressing line, looking for the independent segregation of the introduced traits in the progeny. Traditional breeding work done during the development of the plant line by backcrossing can reveal the linkage of the introduced traits, as well as changes in trait expression. The inheritance data is the ratio of progeny expressing the hemizygous trait based on expected Mendelian inheritance. Stability data implies an examination of either the expression of the trait or tracking of the DNA itself over several plant generations. One of the main concerns with stability is spontaneous loss of the inserted DNA or loss of efficacy due to gene silencing. Neither Corn Event MON 863 nor MON 88017 showed independent assortment of the introduced traits with their marker protein genes (*neomycin phosphotransferase II* and *CP4 enolpyruvylshikimate-3-phosphate*, respectively). This indicates that, in both Corn Event MON 863 and MON 88017, the Cry3Bb1 and marker protein traits were on the same chromosome and closely linked (crossover events were not detected).

d. Protein Characterization and Expression

Data has been presented to demonstrate that the protein expressed from the inserted DNA is similar to what was produced in the source bacterium and is active as expected against the intended target insect. Some protein characterization data demonstrate that microbially produced *Bt* protein is the equivalent to that expressed in the plant. This apparent scientific tautology (where plant-produced protein is the same as microbial protein is the same as the plant-produced protein) has been used to justify the use of the microbially produced protein as a test substance in toxicity tests. Because the expression level of these proteins is so low in plants, and the maximum hazard dose acute oral toxicity test is required as part of the human health risk assessment for these proteins, the ability to produce the protein in an industrial microbe is essential. The acute oral test requires between 2,000 and 5,000 milligrams (mg) of protein per kilogram (kg) bodyweight of test animal. Isolating the amount of purified protein, required to dose several animals, from *Bt*-expressing plants would be a tremendous burden involving harvesting and processing large volumes of plant material (environmental effects testing differs and is addressed in the Environmental Assessment chapter of this Biopesticides Registration Action Document (BRAD)). Proper characterization of the equivalency between these microbial proteins and plant-expressed

proteins provides an alternative to purifying the test material as the plant-produced protein from large volumes of tissue.

Much of the characterization data describes the procedures used to isolate the protein or a highly *Bt* protein-enriched fraction of plant extract. The tests done to support the equivalence of microbial and plant-produced *Bt* protein include the following: molecular sizing by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis; immunorecognition using enzyme-linked immunosorbent assay (ELISA) and western blot analysis; N-terminal amino acid sequencing; matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) analysis of protein digests; confirmation of the lack of glycosylation in the plant-produced protein; and bioactivity against a range of insects (often pest species including the target pest). Since the issues surrounding non-target effects are considered essential for the environmental effects assessment, these non-target pest tests are also discussed in the Environmental Assessment chapter of this BRAD.

The *Bt* protein expression level in various tissues throughout the growing season have been determined for Corn Event MON 863, MON 810 x MON 863, MON 88017, and MON 88017 x MON 810. The data for Corn Event MON 863, however, was presented on a fresh weight basis and data in terms of dry weight leaf, root, pollen, seed, and whole plant were required as a condition of registration. These data (Master Record Identification Number (MRID No.) 464799-02; reviewed in U.S. EPA (2006)) were received and determined acceptable (see section II(A)(2)(b) of this BRAD for a brief summary of these data). Also, to support MON 88017 x MON 810, expression level data regarding Cry1Ab protein levels in MON 810 and MON 88017 x MON 810 young root and forage root were required as a condition of registration. These data (MRID No. 470045-01; reviewed in U.S. EPA (2010a)) were received and determined acceptable (see section II(A)(5)(b) of this BRAD for a brief summary of these data).

e. Residue Analytical Methods

Independent laboratory method validation (Office of Chemical Safety and Pollution Prevention (OCSPP) Harmonized Guideline 860.1340) and EPA laboratory method validation were required to complete the database for Cry3Bb1 corn. The extraction and detection method as described for Cry3Bb1 protein appears to be adequate for analysis of Cry3Bb1 protein in corn grain. The independent laboratory validation study (MRID No. 463942-01; reviewed in U.S. EPA (2006)), required as a condition of registration, was received and found acceptable. Further, in lieu of having the EPA laboratory in Fort Meade, Maryland validate the analytical method for Cry3Bb1, the Agency has confirmed that the Grain Inspection, Packers and Stockyards Administration (GIPSA) performance verification of a qualitative rapid test kit for detecting the presence of Cry3Bb1 in grain and oilseeds has been completed (USDA 2004); thus, the requirement for EPA laboratory method validation for Cry3Bb1 has also been satisfied.

2. Corn Event MON 863 (Organization for Economic Cooperation and Development (OECD) Unique Identifier: ØØ863-5) Expressing Cry3Bb1

a. Data Cited/Submitted for Initial Registration of Corn Event MON 863 (Prior to February 2003)

Cry3Bb1 protein is a delta-endotoxin from *Bacillus thuringiensis* subspecies *kumamotoensis* and has activity against certain beetles. The wild-type *cry*3Bb1 gene was modified to enhance the protein's activity against the corn rootworm complex. Two Cry3Bb1 variants were engineered for expression in the bacterium, *Bacillus thuringiensis* strains EG11098 and EG11231. Cry3Bb1 protein resulting from these strains differed from wild-type Cry3Bb1 protein by 5 and 4 amino acid substitutions (see Table1). Corn was genetically modified to express the Cry3Bb1.11231 protein (resulting in corn line MON 853) or the Cry3Bb1.11098 protein (resulting in corn line MON 863). At the 5' end of the *cry*3Bb1 gene's reading frame, the vectors used for making MON 853 and MON 863 corn coded for an additional amino acid residue due to creation of a restriction enzyme site necessary to construct the vectors.

Table 1. Cry3Bb1 Protein Variants: Amino Acid Sequence Percent Identities and Position Differences

Cry3Bb1 Variant[c]	% Identity Wild-Type	Amino Acid Positions[ab]						
		2	165/166	231/232	311/312	313/314	317/318	348/349
Bacterial-Produced Protein								
Wild-Type	N/A	N/A	D	H	S	N	E	Q
Cry3Bb1.11231	99.4	N/A	D	R	L	T	K	Q
Cry3Bb1.11098	99.2	N/A	G	R	L	T	K	Q
Cry3Bb1.11098 (Q349R)	98.9	A	G	R	L	T	K	R
Cry3Bb1.pvzmir39	99.1	A	D	R	L	T	K	R
Plant-Produced Protein/Product								
Cry3Bb1.11231/ MON 853	99.2	A	D	R	L	T	K	Q
Cry3Bb1.11098 (Q349R)/MON 863	98.9	A	G	R	L	T	K	R
Cry3Bb1.pvzmir39/ MON 88017	99.1	A	D	R	L	T	K	R

[a] A = alanine; G = glycine; D = aspartic acid; R = arginine; H = histidine; L = leucine; S = serine; T = threonine; N = asparagine; K = lysine; E = glutamic acid; Q = glutamine; N/A = not applicable

[b] The *B.t.*-produced Cry3Bb1 protein variants contain 652 amino acids. The *Escherichia coli*- and plant-produced Cry3Bb1 protein variants contain 653 amino acids due to the insertion of an alanine residue at position 2, resulting from the assembly of the *cry*3Bb1 gene into the *E. coli*- or plant-transformation vector.

[c] All Cry3Bb1 protein variants, except for Cry3Bb1.pvzmir39, are discussed in MRID No. 454240-09, while the wild-type Cry3Bb1 is discussed in Donovan *et al*. (1992).

Monsanto Company ("Monsanto") subsequently submitted additional data regarding the MON 863 corn line. The vector, used to transform MON 863 corn, coded for an arginine residue at position 349 instead of glutamine (as previously thought) within the *cry*3Bb1 gene's reading frame. Since the bacterially produced protein used in human health safety studies had the glutamine at position 349 and not arginine (as produced in MON 863), Monsanto generated another package of characterization and toxicology data for this variant, Cry3Bb1.11098 (Q349R). The Agency reviewed the additional data submitted by Monsanto in connection with Corn Event MON 863 and concluded the data support the contention that the Cry3Bb1.11098, Cry3Bb1.11098 (Q349R), and Cry3Bb1.11231 proteins are variants of the Cry3Bb1 protein. Since these variants do not differ significantly from the Cry3Bb1 protein in terms of biochemical or toxicological characteristics, the Cry3Bb1.11098, Cry3Bb1.11098(Q349R), and

Cry3Bb1.11231 protein variants are all covered by the Cry3Bb1 tolerance exemption (40 CFR § 174.518).

The product characterization studies that were submitted in support of Corn Event MON 863 are summarized in Table 2.

Table 2. Product Characterization Data for Corn Event MON 863 (Reviewed in U.S. EPA (2002a) Unless Otherwise Noted).

Study Title	Summary	MRID No.
Data in Support of an Application for Experimental Use Permit for Genetically Modified Corn, Producing a Protein that Provides Control of Corn Rootworm	MON 853, MON 860, MON 862, and MON 863 were produced by the incorporation of one of three constructs (PV-ZMIR12L (MON 862), PV-ZMIR13L (MON 863), or PV-ZMIR14L (MON 853 and MON 860)) via a particle bombardment mechanism. The *cry*3Bb1and *neomycin phosphotransferase II* (*nptII*) genes were stably introduced into the corn genomes, as determined by at least three generations of greenhouse and field studies. **Classification: Acceptable** **(Reviewed in U.S. EPA (2000))**	448779-01
Characterization of *B.t.* Protein 11098 and *B.t.* Protein 11231 Produced by Fermentation	The N-terminal sequence analysis and the immunoreactivity to Cry3Bb1 polyclonal antisera confirm the relationship of Cry3Bb1.11098 and Cry3Bb1.11231 to wild-type Cry3Bb1. Further confirmatory data include protein molecular weight analysis and bioactivity. There are some amino acid changes (four or five) in the two test proteins compared to wild-type. These changes, however, do not appear to significantly affect the bioactivity nor the immunoreactivity of the variant proteins. Based upon the data submitted, the two proteins produced by fermentation—Cry3Bb1.11098 and Cry3Bb1.11231—have been confirmed as Cry3Bb1 protein variants. **Classification: Acceptable**	454240-03
Assessment of the Physiochemical Equivalence of Cry3Bb1.11098 and NPTII Proteins in Corn Event MON 863 to Microbial Sources	Based upon the data provided, it appears that both the Cry3Bb1.11098 and NPTII proteins produced in Event MON 863 have equivalent molecular weights and antigenic properties with these same proteins produced in *B.t.* and *E. coli*, respectively. **Classification: Acceptable** **(MRID No. 451568-03 reviewed in U.S. EPA (2001a))**	451568-03 454240-05

Study Title	Summary	MRID No.
Assessment of the Equivalence of *B.t.* Protein 11098, *B.t.* Protein 11231, and NPTII Protein Expressed in Corn Events MON 853 and MON 860 to Microbial Sources	This report compares the physical (molecular weight, N-terminal sequencing) and functional (bioassay) characteristics of Cry3Bb1.11098 and Cry3Bb1.11231 proteins produced in *E. coli* and corn rootworm (CRW)-protected corn. The data show that the proteins have equivalent molecular weight, immunological reactivities, N-terminal sequences, and comparable median lethal concentration (LC_{50}) values. Furthermore, the data supports the determination of the equivalence of the bacteria- and plant-produced proteins, and the use of the bacterially produced proteins to support registration of the CRW corn product. **Classification: Acceptable**	454240-04
Additional Characterization of the Cry3Bb1 Protein Produced in Corn Event MON 863	Two genetic variants, designated as *cry*3Bb1.11098 and *cry*3Bb1.11231, produce the delta-endotoxin proteins, Cry3Bb1.11098 and Cry3Bb1.11231, respectively. Cry3Bb1.11098 differs from the wild-type *Bacillus thuringiensis* (*B.t.*) protein by 5 amino acids, while the Cry3Bb1.11231 protein differs by 4 amino acids. The *cry*3Bb1.11098 gene was used to develop maize line MON 863 and variant *cry*3Bb1.11231 was used in the development of MON 853 for control of the corn rootworm complex. Further manipulations, during cloning and insertion into the maize genome, brings the total amino acid differences for these two transformants to 7 and 5 for the 11098 (MON 863) and 11231 (MON 853) Cry3Bb1 proteins, respectively. Cry3Bb1 protein was purified from Event MON 863 grain by immunoaffinity chromatography and then analyzed by N-terminal sequencing and MALDI-TOF. Trypsin fragments, subjected to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), provided for identification or verification of 38% of the total protein by mass matching when coupled with sequencing of 29 N-terminal amino acids. Data from MALDI-TOF-MS and N–terminal sequencing indicate that the deduced amino acid sequences of Cry3Bb1.11098, as present in MON 863 and in *B.t.* strain EG11098, are accurate. A comparison of functionality and physicochemical characteristics strongly suggests that the two protein variants are nearly equivalent. Proteins from the fermentation of *B.t.* strains EG11098 and EG11231 were used for mammalian and ecotoxicology studies, as well as in assays relying on immunorecognition of proteins. These proteins are considered as biologically suitable for these studies based upon structural data indicating only minor changes in the shape of the delta-endotoxin proteins. **Classification: Acceptable** **(Reviewed in U.S. EPA (2002b))**	454240-10

Study Title	Summary	MRID No.
Primary Structural Protein Characterization of Corn Event MON 863 Cry3Bb1.11098 Protein Using N-Terminal Sequencing and MALDI-TOF Spectrometric Techniques	Transformation Event MON 863 (maize) produces the 74-kiloDalton (kDa) Cry3Bb1.11098 protein for control of the corn rootworm complex. Modifications to this protein for expression *in planta* bring the differences between the wild-type and MON 863-expressed variant to seven amino acids. Grain from Event MON 863 was used as a source of Cry3Bb1.11098 protein for MALDI-TOF-MS and N-terminal sequence analyses. Of the 653 amino acids present in the 74-kDa form of the Cry3Bb1 protein, 225 were identifiable as to position based upon mass matching. Three fragments, from the N-terminal region of the protein, were also among those matched, representing 43 amino acids. One fragment included the N-terminus, indicating the loss of the terminal methionine and the acetylation of the alanine added at position two. This potentially explains the difficulty in sequencing the N-terminus of the 66-kDa form of the protein eluted from polyvinylidene fluoride (PVDF) blots. Protein samples, obtained from elution off of PVDF membranes of both the 74-kDa and 66-kDa proteins, were subjected to Edman degradation chemistry, but the larger peptide revealed no sequence data, presumably due to blockage of the terminal amino acid residue. When the bacterially produced version of this protein was subjected to N-terminal sequencing procedures, N-terminal sequence data were obtained successfully. The presumed reason for this rests with the post-translational modifications that are typical of eukaryotes (e.g., plants) and are lacking in prokaryotes (e.g., bacteria). Such modification could explain the blockage noted during the attempt to sequence the N-terminus of the corn-derived Cry3Bb1.11098 protein. **Classification: Acceptable** **(Reviewed in U.S. EPA (2002b))**	454240-11

Study Title	Summary	MRID No.
Characterization and Equivalence of the Cry3Bb1 Protein Produced by *E. coli* Fermentation and Corn Event MON 863	MALDI-TOF analysis of the microbial and corn Cry3Bb1.11098 (Q349R) proteins yielded an agreement of 42 to 50 amino acid fragments predicted from the theoretical sequence. The N-terminus of the microbial form lacked the terminal methionine, which is commonly cleaved in expressed proteins. The corn form was apparently not only lacking the terminal methionine, but the N-terminal alanine residue was acetylated as indicated by a 42 Dalton greater weight. The N-terminal amino acid sequence analyses were flawed in that unequivocal determinations were not possible due to the presence of multiple residues in most cycles; however, by comparison to the expected sequences, several different start sites for N-terminal sequencing could be detected. In the *E. coli* Cry3Bb1, the sequence started at both positions 2 and 32. In the corn Cry3Bb1, three different starts were detected at positions 19, 25, and 36. The immunoblot analysis gave similar positive band patterns, indicating the Cry3Bb1 protein produced in both corn and *E. coli* had essentially the same electrophoretic mobility and immunoreactivity. The positive bands were sometimes rather broad (74–66 kDa), but no series of distinct bands could be discerned from the photographs provided. The molecular weight and purity analyses for the corn and microbial extracts indicate that the microbially produced samples were nearly two-fold higher purity in Cry3Bb1 proteins compared to the corn extracts. The purity for Cry3Bb1 was 92.6% and 53.9% for microbial and corn extracts, respectively. Total protein concentrations for the two extracts were determined as 0.58 milligrams per milliliter (mg/mL) and 0.46 mg/mL for microbial and corn extracts, respectively, by colormetric assays. The glycosylation analysis for the Cry3Bb1 extracts gave no positive carbohydrate staining regions for either the microbial or corn samples in the expected regions for Cry3Bb1 protein. The results of the bioassays for the two Cry3Bb1extracts against Colorado potato beetle larvae indicate that there was a dose/response in all tests, and the LC_{50} values were similar and had overlapping 95% confidence intervals. **Classification: Acceptable**	455382-01

Study Title	Summary	MRID No.
B.t. Protein 11231 and NPTII Protein Levels in Samples Collected from Corn Events MON 853 and MON 860 in the 1998 U.S. Field Trials	The protein titer data provided for MON 860 and MON 853 show the ranges of Cry3Bb1 protein in various parts of the plant, as well as geographical variation. Overall, based upon the ranges provided, there appears to be significant variation between the samples analyzed on different days post-planting and at different sites. The registrant mentions a potential difference between decreasing titer in MON 853 and mid-season increasing titer in MON 860. Such a determination, however, cannot be made based upon the data provided in the submission. Even if such a trend was supported by additional data for MON 860, the difference in the protein titers is much smaller than the variation seen for MON 853 on days 44, 55, and 100 post-planting. Ranges of Cry3Bb1 protein levels in MON 853 in microgram Cry3Bb1 protein per gram of fresh weight tissue were 7.01–68.98 (leaf), 1.66–17.64 (root), and 1.23–29.06 (aboveground whole plant). Ranges of Cry3Bb1 protein levels in MON 860 in microgram Cry3Bb1 protein per gram of fresh weight tissue were 32.61–91.11 (leaf), 2.24–10.33 (root), and 0.63–13.95 (aboveground whole plant). **Classification: Acceptable** **(Reference unknown)**	449043-02
Agronomic Equivalency of Corn Event MON 863 Hybrids as Determined in Year 2000 Field Trials	The data included in this submission appear to support the agronomic equivalency of corn Event MON 863 hybrids. Results of the study show that there are some differences in the properties of the transgenic plants versus the control lines used in the tests. Some of the variation identified included differences in corn ear height, plant height, weight, grain moisture, and yield, but in each case, the difference was small. Based upon the data provided, however, it appears that none of these differences would have a significant agronomic impact on the crops and are likely similar to typical differences seen in different plant lines and/or those differences caused by differing ecological effects. **Classification: Acceptable**	453484-03
Molecular Analysis of Corn Event MON 863	The data presented in this submission describe the DNA insert for Event MON 863. The data provided support the finding that Event MON 863 contains 1 intact copy of the insert that encodes for both Cry3Bb1 and NPTII proteins. **Classification: Acceptable**	454240-02 451568-01
B.t. Cry3Bb1.11098 and NPTII Protein Levels in Tissue Samples Collected from Corn Event MON 863 Grown in 1999 Field Trials	The protein titer data provided show the ranges of Cry3Bb1 protein in various parts of the plant, as well as geographical variation. Overall, based upon the ranges provided, there appears to be significant variation between the samples analyzed on different days post-planting and at different sites. Ranges of Cry3Bb1 protein levels in MON863 in microgram Cry3Bb1 protein per gram of fresh weight tissue were 30–93 (leaf), 49–86 (grain), 30–93 (pollen), 3.2–66 (root), and 13–54 (aboveground whole plant). **Classification: Acceptable**	454240-01 451568-02

Study Title	Summary	MRID No.
Validated Method for Extraction and Direct ELISA Analysis of Cry3Bb1 in Corn Grain	The extraction and detection method, as described for Cry3Bb1 protein, uses Cry3Bb1-specific antisera for direct sandwich ELISA of Cry3Bb1 protein in corn grain. This ELISA method does not involve a commercially available test kit (with pre-coated wells) and is not intended to be used as an enforcement method. The EPA laboratory (Fort Meade, Maryland) has concluded that this work was done early in the development of the biotech event and represents research that led to the implementation of the test kits. This method is not suitable for method validation as it is not a practical method to be used to routinely evaluate field samples. **(Reviewed in U.S. EPA (2007))**	453731-01

b. Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)

When Corn Event MON 863 (EPA Reg. No. 524-528) was initially registered on February 24, 2003, the Agency issued a registration notice to Monsanto that contained the following requirements for further product characterization information:

> "Submit independent laboratory method validation (under OPPTS Guidelines 860.1340) to complete the database for Cry3Bb1 corn within 12 months of the date of registration. Provide the EPA laboratory (Fort Meade, MD) methodology and/or reagents necessary for validation of a Cry3Bb1 analytical method within 6 months of the date of registration. The extraction and detection method as described for Cry3Bb1 protein appears to be adequate for analysis of Cry3Bb1 protein in corn grain. However, this method must be validated by both an independent laboratory and the EPA Biological and Economic Analysis Division laboratory before it can be considered a valid method…."
>
> "Submit expression data in terms of dry weight, as the amount of protein present in the given tissue. Tissues for which expression data must be provided include: leaf, root, pollen, seed, and whole plant. In addition, data for each of these tissues should be provided for young plants in rapid growth, during flowering, and mature plants before harvest when that part of the plant is present. Data obtained for roots should also include typical times when corn rootworm when be feeding. Data are due within 24 months of the date of registration…."

For the Corn Event MON 863 registration, the abovementioned requirements for additional product characterization data have been satisfied by submission of appropriate studies and information; summaries of this information are presented in Table 3.

Table 3. Product Characterization Data for Corn Event MON 863 (Reviewed in U.S. EPA (2006)).

Study Title	Summary	MRID No.
TraitChek™ Cry3Bb1 Lateral Flow Test Strip and SeedChek™ Cry3Bb1 ELISA Performance Verification for Corn Seed, Leaf, and Composite Testing	The TraitChek™ Cry3Bb1 lateral flow strip test and the SeedChek ™ Cry3Bb1 ELISA were evaluated for qualitative detection of Cry3Bb1 protein in corn seed and leaves. Three lots of each test kit were used to evaluate each of 100 known Cry3Bb1 corn seed or corn leaf samples and 100 non-transgenic control samples. In single-seed testing of known Cry3Bb1 kernels, both tests were 100% accurate. In composite-seed testing (1 known Cry3Bb1 kernel in 800 total kernels), the TraitChek™ test had a false negative rate of 0.3% after a five-minute reading but was 100% accurate after ten minutes. The SeedChek ™ test was 100% accurate at both reading times. Both tests showed 100% accuracy in detecting Cry3Bb1 in leaf samples from 13 varieties of Cry3Bb1 corn. No cross-reactivity with other purified recombinant proteins or corn events expressing other recombinant proteins was observed for either test. **Classification: Acceptable** *Note for 2010: Although the requirement for an independent laboratory validation of the Cry3Bb1 analytical method was satisfied by submission of this information (MRID No. 463942-01), the reviewer also concluded that "EPA's Analytical Method Laboratory located in Fort Meade (Maryland) will have to independently validate Monsanto's lateral flow strip test and ELISA protocol for accuracy, precision, and sensitivity." More recently, the Agency has decided to allow this particular requirement to be satisfied by the GIPSA performance verification of a qualitative rapid test kit for detecting the presence of the biotechnology event in grains and oilseeds (USDA 2004). In the case of Cry3Bb1, the Agency has now confirmed that a test kit has been verified by GIPSA and, therefore, the science reviewer's concern has been addressed, and the original condition has been satisfied for the Corn Event MON 863 registration.	463942-01

Study Title	Summary	MRID No.
Cry3Bb1 and NPTII Protein Levels in Corn Tissues from MON 863 Produced in 2003 U.S. Field Trials	The levels of proteins Cry3Bb1 and NPTII were determined in tissues of transgenic corn MON 863 generated in five 2003 U.S. field trials. The two proteins were quantitated by validated ELISA and presented in dry tissue weight (dwt). The highest Cry3Bb1 levels were in leaf, whole plant, and root samples, which had, respectively, mean levels of 180–240, 130–340, and 140–290 micrograms per gram (µg/g) dwt. Levels of Cry3Bb1 tended to be lower at the V10–V12 growth stage in whole plants and roots than for the earlier stages (V2–V3, V4–V5, V6–V7). Forage and forage root had similar Cry3Bb1 levels (mean of 55 and 80 µg/g dwt, respectively), and the lowest Cry3Bb1 levels were in pollen, stover, senescent root, and grain (mean of 20–35 µg/g dwt). NPTII protein was only measured in grain, where it was not detected. The frozen storage stability of Cry3Bb1 protein was not established for any unprocessed tissues or for processed whole plant, forage, stover, pollen, and grain samples. The storage stability of the NPTII protein was unknown for processed grain. Storage stability, however, was established for root tissue within the time frame it was analyzed, which is the most important factor in determining dose/susceptibility for pests. This is because the susceptible pest species, corn rootworm larvae, most actively feeds on root tissue at this stage of plant development. Thus, it is important that the protein expression levels, in particular root tissues, be accurately established to demonstrate the efficacy of the plant-incorporated protectant (PIP) plant. Moreover, Monsanto states that the levels of Cry3Bb1 from MON 863 were consistent with previously submitted tissue expression data (from MON 863 corn grown in 1999 field trials). Therefore, although some samples were analyzed outside the demonstrated storage stability time frame, it is unlikely that these samples were compromised in -80°C storage. **Classification: Acceptable**	464799-02

3. MON 863 x MON 810 (OECD Unique Identifier: MON-ØØ863-5 x MON- ØØ81Ø-6) Expressing Cry3Bb1 and Cry1Ab

a. Data Cited/Submitted for Initial Registration of MON 863 x MON 810 (Prior to October 2003)

MON 863 x MON 810 (also known as YieldGard® Plus Corn) was developed by crossing inbred lines of Event MON 863, which expresses the Cry3Bb1 and NPTII proteins, and Event MON 810, which expresses the Cry1Ab protein. This product is considered a stacked plant-incorporated protectant because it targets both lepidopteran and coleopteran pests and contains two separate active ingredients. MON 810 is briefly summarized in the paragraph that follows, while additional information on MON 863, specifically related to product characterization, can be found in section II(A)(2) of this BRAD.

MON 810 produces *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1 Cry1Ab protein to selectively control larvae of European corn borer (*Ostrinia nubilalis*) and other lepidopteran pests. On December 20, 1996, the Agency issued a FIFRA section 3 registration to Monsanto for MON 810 (EPA Reg. No. 524-489), expressing *Bacillus thuringiensis* Cry1Ab protein and the genetic material necessary (PV-ZMCT01) for its production in corn Event MON 810 (OECD Unique Identifier: MON-ØØ81Ø-6). The product characterization data supporting the registration of MON 810, including the submitted study titles, conclusions, and their MRID Numbers, can be found in both the 2001 *Bt* Crops Reassessment and the September 2010 Cry1Ab and Cry1F BRAD (U.S. EPA 2001b, 2010b).

The data submitted for MON 863 x MON 810, which include DNA analysis of the inserts in and protein expression data for the stacked corn product, are summarized in Tables 4 and 5.

Table 4. Product Characterization Data for MON 863 x MON 810 (Reviewed in U.S. EPA (2003)).

Study Title	Summary	MRID No.
Confirmation of the Molecular Identity of YieldGard and Corn Rootworm-Protected Combined Trait Corn Hybrid MON 810 x MON 863 by Southern Blot Analysis	The corn hybrid, MON 810 x MON 863, containing the two transformation events, MON 810 (*cry*1Ab) and MON 863 (*cry*3Bb1), was examined for the presence of these two genes encoding delta-endotoxins in the resulting hybrid. Probes for the *cry*1Ab and *cry*3Bb1 genes were obtained from previous studies and corresponded to the first 900 base pairs (*cry*1Ab) or the entire length of the gene (*cry*3Bb1). The radiolabeled (^{32}P) probe for *cry*1Ab hybridized to restricted DNA samples on nylon membranes and resulted in a lack of any signal detection for the samples from MON 846 (non-transgenic) and MON 863 but did detect the presence of *cry*1Ab in the MON 810 and MON 810 x MON 863 hybrid plant samples. When DNA samples were probed with the *cry*3Bb1 sequence, hybridization confirmed the presence of this gene in MON 863 plants and the MON 810 x MON 863 hybrid, but again failed to detect the presence of this gene in the negative control, MON 846. Plasmid DNA from plasmids containing either gene separately did react positively with the appropriate probes when the restricted plasmid DNA was co-electrophoresed with MON 846 DNA and hybridized with the respective probe. From these results, it is evident that MON 810 x MON 863 contains the *cry*1Ab and *cry*3Bb1 genes. Additionally, the restriction patterns noted on the Southern blot suggest that there have been no major alterations or rearrangements in the conventional cross of these two events (hybrids) for these two gene inserts. **Classification: Acceptable**	457917-01

Study Title	Summary	MRID No.
Cry3Bb1, Cry1Ab and NPTII Protein Levels in the Dual-Trait Maize Hybrid MON 863 x MON 810 Produced in Argentina Field Trials Conducted During the 1999–2000 Growing Season	ELISA values for field-grown maize samples (grain, forage, root, leaf, and pollen) were examined with a double antibody sandwich technique. Cry3Bb1 and NPTII protein values were adjusted for method bias to optimize the accuracy based upon extraction efficiency, and for variance in storage stability of extracts in some instances. The ranges of protein levels observed for Cry3Bb1, Cry1Ab, and NPTII were similar across all four sites for the MON 863 x MON 810 hybrid and the MON 863 or MON 810 hybrids as appropriate for the transgene in question. Averages for the Cry3Bb1 and Cry1Ab proteins were, however, somewhat higher in the MON 863 x MON 810 hybrid versus the single trait hybrids. NPTII protein levels and ranges in the MON 863 x MON 810 and MON 863 hybrids were similar. The highest levels of expression of Cry3Bb1 or Cry1Ab were found to occur in pollen for Cry3Bb1 (79.6 µg/g fresh weight) and in leaf (17.9 µg/g fresh weight) for Cry1Ab. **Classification: Acceptable**	457917-02

Table 5. Comparison of Cry1Ab and Cry3Bb1 Protein Levels in Dual-Trait and Single-Trait Hybrids

	Average Cry1Ab Protein Levels (µg/g fwt) (Range)		Average Cry3Bb1 Protein Levels (µg/g fwt) (Range)	
Tissue Type and Collection Time (Days Post-Planting)	**MON 863 x MON 810**	**MON 810**	**MON 863 x MON 810**	**MON 863**
Young Leaf (≈18)	17.9 (14.1–27.5)	13.0 (9.8–15.4)	46.7 (35.5–53.2)	30.0 (21.3–47.2)
Forage (≈90)	7.9 (3.9–11.9)	5.6 (3.0–8.2)	23.6 (6.7–39.7)	12.8 (<0.22–28.8)
Grain (≈117)	0.84 (0.63–1.2)	0.46 (0.24–0.77)	61.1 (38.5–83.1)	43.7 (<0.096–84.1)
Pollen (≈60)	<0.08 (<0.08–0.18)	<0.08 (<0.08)	79.6 (65.1–96.5)	60.4 (29.7–90.7)
Mature Root (≈90)	N/A	N/A	19.7 (6.0–41.7)	16.2 (<0.76–49.8)
Over-Season Root (≈46)	N/A	N/A	22.0 (N/A)	20.0 (N/A)

fwt= fresh weight
N/A= Not Applicable
Data from MRID No. 457917-02, pages 10–11

b. Terms and Conditions of the MON 863 x MON 810 Registration (October 2003 – September 2010)

When MON 863 x MON 810 (EPA Reg. No. 524-545) was initially registered on October 31, 2003, the Agency issued a registration notice to Monsanto that contained the following requirement:

> "Submit all data required to support the individual plant-incorporated protectants in MON 810 and MON 863 corn, EPA Registration Nos. 524-489 & 524-528."

All requirements for additional product characterization information for MON 810 (see U.S. EPA (2010b)) and MON 863 (see section II(A)(2)(b) of this BRAD) have been satisfied for the purposes of this registration.

4. MON 88017 (OECD Unique Identifier: MON-88Ø17-3) Expressing Cry3Bb1

a. Data Cited/Submitted for Initial Registration of MON 88017 (Prior to December 2005)

The Cry3Bb1 protein, as produced in MON 88017, is a variant of the wild-type Cry3Bb1 protein from *Bt* subsp. *kumamotoensis*, and it protects the roots of corn plants from feeding damage caused by the coleopteran pest, corn rootworm. The amino acid sequence of the Cry3Bb1.pvzmir39 variant (MON 88017) differs by seven amino acids from the wild-type Cry3Bb1 protein and by a single amino acid from the Cry3Bb1.11098 (Q349R) protein (MON 863). The Cry3Bb1 protein variants in MON 88017 and MON 863 share an amino acid sequence identity of >99.8%, differing from one another by only 1 of 653 amino acids at position 166 in MON 863, where glycine is present instead of aspartic acid (see Table 1 in section II(A)(2)(a) of this BRAD). MON 88017 also expresses the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* spp. strain CP4 (CP4 EPSPS), which confers tolerance to glyphosate (Roundup®) herbicides.

All physicochemical characteristics (including immunoreactivity, amino acid sequence, molecular weight, and glycosylation status) in the Cry3Bb1 protein in MON 88017 were found to be similar with the Cry3Bb1 protein in MON 863. Moreover, the protein expression, functional activity, and field efficacy data of Cry3Bb1 protein in MON 88017 were compared to MON 863 and found to be functionally equivalent.

The product characterization studies that were submitted in support of MON 88017 are summarized in Table 6.

Table 6. Product Characterization Data for MON 88017 (Reviewed in U.S. EPA (2005a)).

Study Title	Summary	MRID No.
Molecular Analysis of YieldGard Rootworm/Roundup Ready Corn Event MON 88017	Corn Event MON 88017 contains one copy of the transfer deoxyribonucleic acid (T-DNA) at a single integration locus on an approximately 13 kilobase *Sca* I restriction fragment. The plasmid vector contains the *cry3Bb1.pvzmir39* and *cp4 epsps* expression cassettes within the borders. No additional elements from the transformation vector PV-ZMIR39 or plasmid backbone sequences were detected in the corn genome of MON 88017. Insert stability was confirmed over multiple generations. PCR and DNA sequence analyses confirmed the organization of the elements within the insert and determined the complete DNA sequence of the insert in corn Event MON 88017. These data confirm that only the two expected full-length proteins, Cry3Bb1 and CP4 EPSPS, are encoded by the insert in corn Event MON 88017. **Classification: Acceptable**	461817-02 465783-01
Cry3Bb1 and CP4 EPSPS Protein Levels in Corn Tissues Collected from MON 88017 Corn Produced in U.S. Field Trials Conducted in 2002	Tissue samples were collected at various times throughout the growing season from MON 88017 corn grown in U.S. field trials at three field sites in 2002. Tissue samples were analyzed for Cry3Bb1 and CP4 EPSPS protein levels using validated ELISA methods. The mean Cry3Bb1 levels across three field sites for leaf, whole plant, and root tissues harvested throughout the growing season ranged from 260–570, 88–500, and 100–370 µg/g dry tissue weight, respectively. The mean Cry3Bb1 protein levels for pollen, forage, silk, and grain tissue were 25, 95, 380, and 15 µg/g dry tissue weight, respectively. The mean CP4 EPSPS protein levels for leaf and root tissue throughout the growing season ranged from 150–220 and 70–150 µg/g dry tissue weight, respectively. The mean CP4 EPSPS protein levels for pollen, forage, and grain tissue were 390, 57, and 5.8 µg/g dry tissue weight, respectively. CP4 EPSPS protein levels were not assessed in whole plant or silk tissue. These data establish the protein levels of Cry3Bb1 and CP4 EPSPS proteins on a fresh weight and dry weight basis in the various tissues throughout the growing season. **Classification: Acceptable**	461817-03 465783-02

Study Title	Summary	MRID No.
Evaluation of the Functional Equivalence of Two Cry3Bb1 Protein Variants Against Susceptible Coleopteran Species	Both Colorado potato beetle (CPB) and western corn rootworm (WCRW) diet bioassays indicate that there are no significant differences in the biological activity between the two protein variants, Cry3Bb1.11098 (Q349R) produced in MON 863 and Cry3Bb1.pvzmir39 produced in MON 88017. The two protein variants are functionally equivalent in biological activity against the susceptible coleopteran insect species, CPB and WCRW. The mean dietary median lethal concentrations for CPB larvae were 0.95 micrograms per milliliter (µg/mL) (ranging from 0.79–1.11 µg/mL) for the Cry3Bb1.11098 (Q349R) protein and 0.84 µg/mL (ranging from 0.64–1.06 µg/mL) for diets containing the Cry3Bb1.pvzmir39 protein. For WCRW larvae, the mean dietary median lethal concentrations were 100 µg/mL (ranging from 73.2–137 µg/mL) for the Cry3Bb1.11098 (Q349R) protein and 139 µg/mL (ranging from 74.6–231 µg/mL) in diets containing the Cry3Bb1.pvzmir39 protein. **Classification: Acceptable**	461817-04 465783-03

b. Terms and Conditions of the MON 88017 Registration (December 2005 – September 2010)

When MON 88017 (EPA Reg. No. 524-551) was initially registered on December 13, 2005, the Agency issued a registration notice to Monsanto that contained the following requirement:

> "Submit all data required to support the individual plant-incorporated protectant in Event MON863 (YieldGard Rootworm), 524-528…."

All requirements for additional product characterization information for MON 863 (see section II(A)(2)(b) of this BRAD) have been satisfied for the purposes of this registration.

5. MON 88017 x MON 810 (OECD Unique Identifier: MON-88Ø17-3 x MON-ØØ81Ø-6) Expressing Cry3Bb1 and Cry1Ab

a. Data Cited/Submitted for Initial Registration of MON 88017 x MON 810 (Prior to December 2005)

The stacked product, MON 88017 x MON 810, is a hybrid created by crossing the individuals events, MON 88017 (Cry3Bb1) and MON 810 (Cry1Ab), via traditional breeding methods. Product characterization background on MON 88017 was discussed in section II(A)(4) of this BRAD; MON 810 was briefly summarized in section II(A)(3)(a), but more comprehensive information can be found in either the 2001 *Bt* Crops Reassessment or the September 2010 Cry1Ab and Cry1F BRAD (U.S. EPA 2001b, 2010b).

The data submitted for MON 88017 x MON 810, which include molecular characterization data and protein expression analyses, are summarized in Table 7.

Table 7. Product Characterization Data for MON 88017 x MON 810 (Reviewed in U.S. EPA (2005b)).

Study Title	Summary	MRID No.
Confirmation of the Identity of MON 88017 x MON 810 Corn by Southern Blot Analysis	Southern blot fingerprints confirmed the presence of the event-specific fingerprints for MON 88017 and MON 810 in the Roundup Ready® hybrid, MON 88017 x MON 810. The fingerprints of MON 88017 x MON 810 were consistent with previously reported event-specific fingerprints for MON 88017 and MON 810, which produce the Cry3Bb1 and Cry1Ab proteins, respectively. **Classification: Acceptable**	461850-02
Cry3Bb1,CP4 EPSPS, and Cry1Ab Protein Levels in Corn Tissues Collected from MON 88017 x MON 810 Corn Produced in U.S. Field Trials Conducted in 2002	Tissue samples were collected at various times throughout the growing season from MON 88017 x MON 810 corn grown during 2002 at three field sites in the U.S. Tissue samples were analyzed for Cry3Bb1, CP4 EPSPS, and Cry1Ab protein levels using validated ELISA methods. The levels of Cry3Bb1 and Cry1Ab protein in tissue samples from the control hybrid were below the limit of quantitation (LOQ) or limit of detection (LOD) for each tissue assay. The mean Cry3Bb1 protein levels in MON 88017 x MON 810 corn for root tissue harvested at the V2–V3 stage ranged from 140–350 µg/g dwt. The mean Cry3Bb1 protein levels for leaf, pollen, grain, and forage tissues were 670, 27, 9.3, and 100 µg/g dwt, respectively. The mean CP4 EPSPS protein levels in MON 88017 x MON corn for grain and forage tissues were 4.3 and 51 µg/g dwt, respectively. Finally, the mean Cry1Ab protein levels in MON 88017 x MON 810 corn for leaf, grain, and forage tissues were 110, 0.39, and 14µg/g dwt, respectively. The mean Cry1Ab protein level for pollen was below the limit of detection (LOD, 0.090µg/g fwt). The results demonstrate that the range and mean Cry3Bb1 and Cry1Ab expression levels in each tissue of MON 88107 x MON 810 were similar to the corresponding levels in the single-trait events, MON 88017 and MON 810, respectively. Finally, the levels of the inert ingredient, CP4 EPSPS protein, produced in MON 88017 x MON 810 were similar to the levels observed for the same CP4 EPSPS protein produced in MON 88017. **Classification: Acceptable**	461850-03

b. Terms and Conditions of the MON 88017 x MON 810 Registration (December 2005 – September 2010)

When MON 88017 x MON 810 (EPA Reg. No. 524-552) was initially registered on December 13, 2005, the Agency issued a registration notice to Monsanto that contained the following requirements:

> "Submit all data required to support the individual plant-incorporated protectants in MON 810 (YieldGard), Event MON863 (YieldGard Rootworm)…, EPA Registration Nos. 524-489, 524-528…."

"Submit expression level data regarding Cry1Ab protein levels in MON 810 and MON 88017 x MON 810 young root and forage root within 12 months of the date of registration."

All requirements for additional product characterization information for MON 810 (see U.S. EPA (2010b)) and MON 863 (see section II(A)(2)(b) of this BRAD) have been satisfied for the purposes of this registration. For the MON 88017 x MON 810-specific conditional data requirement (i.e., expression level data), a study was submitted to the Agency and found acceptable (see Tables 8 and 9).

Table 8. Product Characterization Data for MON 88017 x MON 810 (Reviewed in U.S. EPA (2010a)).

Study Title	Summary	MRID No.
Assessment of Cry1Ab Protein Levels in Corn MON 88017 x MON 810 Root Tissue Produced in U.S. Field Trials in 2006	A traditionally crossed corn hybrid of MON 88017 with MON 810 was grown along with conventional seed and MON 810 corn at five locations in 2006 using a randomized complete block design and sampling scheme. Young root tissues were sampled at V2–V3 and forage root tissues at early dent or 1/3 milkline. Samples were stored and shipped on dry ice for Cry1Ab analysis of trypsinized, extracted tissues. Extraction efficiency was 92%, spike recovery was 77%, and the trypsinization factor was 2. The coefficient of variation was 14% between assays. Limit of detection was 0.13 µg/g fresh weight, and limit for quantification was 0.40 µg/g fresh weight. ELISA revealed mean Cry1Ab protein levels in MON 88017 x MON 810 corn tissues across all sites were 75 µg/g dwt in young root and 12 µg/g dwt in forage root; similar to the mean Cry1Ab protein levels in MON 810 corn, which were 78 µg/g dwt in young root and 13 µg/g dwt in forage root. **Classification: Acceptable**	470045-01

Table 9. Cry3Bb1 and Cry1Ab Protein Levels in MON 88017 x MON 810 and MON 88017 & MON 810 Tissues.

Tissue Type	Growth Stage	Cry3Bb1 Protein Levels (µg/g dwt)						Cry1Ab Protein Levels (µg/g dwt)					
		MON 88107 x MON 810			MON 88017			MON 88017 x MON 810			MON 810		
		Mean	Standard Deviation	Range n=9	Mean	Standard Deviation	Range n=9	Mean	Standard Deviation	Range n=9	Mean	Standard Deviation	Range n=9
Young Leaf	V2–V3	670	130	550-920	570	170	230-820	110	17	85-140	100	12	89-130
Young Root	V2–V3	350	150	88-560	370	80	240-510	75*	26*	14-130*	78*	21*	51-130*
Pollen	R1	27	5.7	N/A-34	25	4.2	17-32	N/A	N/A	N/A	N/A	N/A	N/A
Forage	R4–R6 (early dent)	100	23	71-150	95	19	75-130	14	2.1	11-17	14	3.4	8.4-19
Forage Root	R4–R6 (early dent)	140	29	89-180	130	29	98-170	12*	4.7*	2.7-22*	13*	5.1*	6.6-21*
Grain	R6	9.3	3.4	3.9-13	15	3.6	10-22	0.39	0.13	0.16-0.63	0.43	0.091	0.27-0.54

dwt= dry weight
N/A= not applicable (as levels were below the level of detection)
Data from MRID No. 461850-01, page 14
*Data from MRID No. 470045-01

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B. Human Health Assessment

1. Background

The basic premise relied on for the toxicology assessment is the fact that all the *Bacillus thuringiensis* (*Bt*) plant-incorporated protectants are proteins. Proteins are commonly found in the diet and, except for a few well described phenomena, present little risk as a mammalian hazard.

Several types of data are required for the *Bt* plant-incorporated protectants to provide a reasonable certainty that no harm will result from the aggregate exposure to these proteins. The information is intended to show that the *Bt* protein behaves as would be expected of a dietary protein, is not structurally related to any known food allergen or protein toxin, and does not display any oral toxicity when administered at high doses. These data consist of an *in vitro* digestion assay, amino acid sequence homology comparisons, and an acute oral toxicity test. The acute oral toxicity test is done at a maximum hazard dose using purified protein of the plant-incorporated protectant as a test substance. Due to limitations of obtaining sufficient quantities of pure protein test substance from the plant itself, an alternative production source of the protein, such as the *Bt* source organism or an industrial fermentation microbe, is often used. The justification for employing this alternative source of pure protein is the equivalence data discussed section II(A)(2)(a) of this Biopesticides Registration Action Document (BRAD).

In general, the Environmental Protection Agency (EPA) believes that protein instability in digestive fluids and the lack of adverse effects using the maximum hazard dose approach eliminate the need for longer term testing of *Bt* protein plant-incorporated protectants. Dosing of animals with the maximum hazard dose, along with product characterization data, should identify potential toxins and allergens and provide an effective means to determine the safety of these proteins. The adequacy of the current testing requirements was discussed at the June 7, 2000 Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) meeting. In their final report, the SAP agreed in principle with the methods used by EPA to assess the toxicity of proteins expressed in plants, especially the maximum hazard dose approach (U.S. EPA 2000).

a. *In vitro* Digestibility Assay

The intent of this assay is to demonstrate that the *Bt* protein is degraded into small peptides or amino acids in solutions that mimic digestive fluids. Usually, only gastric fluid is tested since Cry protein is known to be stable in intestinal fluid. In order to track the breakdown, the proteins were added to a solution of the digestive fluids, and a sample was either removed or quenched at given time points (usually at time 0, one to several minutes later, and one hour later). The time-point samples were then electrophoresed on either a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and further analyzed by western blot or tested in a bioassay against the target pest.

As has been stated in several public fora, the *in vitro* digestibility test is basically a test to confirm the biochemical characteristic of instability of the protein in the presence of digestive fluids. The digestibility test is not intended to provide information on the toxicity of the protein or imply that similar breakdown will happen in all human digestive systems. The *in vitro* digestibility assay may also provide information about the potential of a protein to be a food allergen. The *in vitro* digestion assays confirm that the protein is being broken down in the presence of typical digestive fluids and is not unusually persistent in the digestive system. One of the limitations of the test is that it usually only tracks protein breakdown to fragments still recognized by the immunological reagents employed.

b. Amino Acid Homology

An additional characteristic that is considered as an indication of possible relation to a food allergen is a protein's amino acid sequence when compared to known food allergens.

c. Acute Oral Toxicity

One of the bases for addressing the toxicity of proteins primarily through the use of acute oral toxicity is that, when demonstrated to be toxic, proteins are toxic at low doses (Sjoblad *et al*. 1992). Therefore, when no effects are shown to be caused by the protein plant-incorporated protectants, even at relatively high-dose levels in the acute oral exposure test, the proteins are not considered toxic.

2. Human Health Assessment of Cry3Bb1

The detailed Agency human health assessment of Cry3Bb1 corn is found in U.S. EPA (2002a). Portions of the data used in the human health assessment are reviewed in U.S. EPA (2001a, 2001b, 2001c, 2001d, 2001e, and 2005a). A summary of the key findings is provided below.

a. Mammalian Toxicity and Allergenicity Assessment

Based upon the human health data provided, there is minimal risk of toxic and/or allergenic effects to humans or animals due to exposure to the Cry3Bb1 proteins. Based on review of the data, there is a reasonable certainty of no harm to humans and animals posed by the aggregate exposure to residues of these proteins.

i. Mammalian Toxicity

Data have been submitted demonstrating the lack of mammalian toxicity at high levels of exposure to the pure Cry3Bb1 proteins. These data demonstrate the safety of the products at levels well above maximum possible exposure levels that are reasonably anticipated in the crops. Basing this conclusion on acute oral toxicity data without requiring further toxicity testing and residue data is similar to the Agency position regarding toxicity and the requirement of residue data for the microbial *Bt* products from which these plant-incorporated protectants were derived (see 40 CFR §§ 158.2130(d)(1)(i) and 158.2140(d)(7)). For microbial products, further toxicity testing and residue data are triggered by

significant acute effects in studies, such as the mouse oral toxicity study, to verify and quantify the observed effects and clarify the source of these effects (Tiers II and III).

Three acute oral studies were submitted for the Cry3Bb1 proteins (Master Record Identification Numbers (MRID Nos.) 449043-05, 449043-06, and 455382-02; reviewed in U.S. EPA (2001a, 2001b, and 2002a)). These studies were done with three variants of the Cry3Bb1 protein engineered with either four or five internal amino acid sequence changes to enhance activity against the corn rootworm. The acute oral toxicity data submitted support the prediction that the Cry3Bb1 protein would be non-toxic to humans. Male and female mice (10 of each) were dosed with 36, 396, or 3,780 milligrams/kilograms bodyweight (mg/kg bwt) of Cry3Bb1 protein for one variant. The mice were dosed with 38.7, 419, or 2,980 mg/kg bwt of Cry3Bb1 protein for the second variant. The mice were dosed with 300, 900, or 2,700 mg/kg bwt of Cry3Bb1 protein for the third variant. In one study, two animals in the high-dose group died within a day of dosing. These animals both had signs of trauma, probably due to dose administration (i.e., lung perforation or severe discoloration of lung, stomach, brain, and small intestine). No clinical signs were observed in the surviving animals and body weight gains were recorded throughout the 14-day study for the remaining animals. Gross necropsies indicated no findings of toxicity attributed to exposure to the test substance in any of the three studies. No other mortality or clinical signs attributed to the test substance were noted during any study.

When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Sjoblad *et al*. 1992). Therefore, since no effects were shown to be caused by the plant-incorporated protectants, even at relatively high dose levels, the Cry3Bb1 proteins are not considered toxic. Further, amino acid sequence comparisons showed no similarity between the Cry3Bb1 proteins and known toxic proteins available in public protein databases.

ii. Allergenicity Assessment

Since Cry3Bb1 is a protein, allergenic sensitivities were considered. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by acid and proteases, may be glycosylated, and can be present at high concentrations in the food.

Data have been submitted that demonstrate that the Cry3Bb1 proteins are rapidly degraded by gastric fluid *in vitro*. In a solution of simulated gastric fluid (pH 1.2), complete degradation of detectable Cry3Bb1 protein occurred within 30 seconds. Insect bioassay data indicated that the protein lost insecticidal activity within 2 minutes of incubation in simulated gastric fluid (SGF). Incubation in simulated intestinal fluid resulted in a protein digestion product of ~59 kiloDaltons (kDa). A comparison of amino acid sequences of known allergens uncovered no evidence of any homology with Cry3Bb1, even at the level of 8 contiguous amino acids residues.

iii. Conclusion

The potential for the Cry3Bb1 proteins to be food allergens is minimal. Regarding toxicity to the immune system, the acute oral toxicity data submitted support the prediction that the Cry3Bb1 proteins would be non-toxic to humans. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Sjoblad *et al*. 1992). Therefore, since no effects were shown to be caused by the plant-incorporated protectants, even at relatively high dose levels, the Cry3Bb1 proteins are not considered toxic.

b. Aggregate Exposures

In examining aggregate exposure, section 408 of the Federal Food, Drug, and Cosmetic Act (FFDCA) directs EPA to consider available information concerning exposures from the pesticide residue in food and all other non-occupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

The Agency has considered available information on the aggregate exposure levels of consumers (and major identifiable subgroups of consumers) to the pesticide chemical residue and to other related substances. These considerations include dietary exposure under the tolerance exemption and all other tolerances or exemptions in effect for the plant-incorporated protectants' chemical residue, and exposure from non-occupational sources. Exposure via the skin or inhalation is not likely since the plant-incorporated protectants are contained within plant cells, which essentially eliminates these exposure routes or reduces these exposure routes to negligible. Oral exposure, at very low levels, may occur from ingestion of processed corn products and, potentially, drinking water. However, a lack of mammalian toxicity and the digestibility of the plant-incorporated protectants have been demonstrated. The use sites for the Cry3Bb1 proteins are all agricultural for control of insects. Therefore, exposure via residential or lawn use to infants and children is not expected. Even if negligible exposure should occur, the Agency concludes that such exposure would present no risk due to the lack of mammalian toxicity and low potential for allergenicity demonstrated for the Cry3Bb1 proteins.

c. Cumulative Effects from Substances with a Common Mechanism of Toxicity

Section 408(b)(2)(D)(v) of FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider "available information concerning the cumulative effects of [a particular pesticide's] residues and other substances that have a common mechanism of toxicity."

EPA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Because there is no indication of mammalian toxicity resulting from the plant-incorporated protectants, the Agency concludes that there are no cumulative effects for the Cry3Bb1 proteins. For information regarding EPA's efforts to determine which chemicals have a common mechanism of

toxicity and to evaluate the cumulative effects of such chemicals, see EPA's website at *http://www.epa.gov/pesticides/cumulative*.

d. Determination of Safety for U.S. Population, Infants, and Children

i. Toxicity and Allergenicity Conclusions

The data submitted and cited regarding potential health effects for the Cry3Bb1 proteins include the characterization of the expressed Cry3Bb1 proteins in corn, as well as the acute oral toxicity and *in vitro* digestibility of the proteins. The results of these studies were determined applicable to evaluate human risk, and the validity, completeness, and reliability of the available data from the studies were considered.

Adequate information was submitted to show that the Cry3Bb1 protein test material derived from microbial cultures was biochemically and functionally similar to the protein produced by the plant-incorporated protectant ingredients in corn. Production of microbially produced protein was chosen in order to obtain sufficient material for testing.

The submitted acute oral toxicity data support the prediction that the Cry3Bb1 proteins would be non-toxic to humans. As mentioned in section II(B)(2)(a)(i) of this BRAD, when proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Sjoblad *et al*. 1992). Since no effects were shown to be caused by the Cry3Bb1 proteins, even at relatively high dose levels (3,780 mg/kg bwt of Cry3Bb1 protein), the Cry3Bb1 proteins are not considered toxic. Basing this conclusion on acute oral toxicity data without requiring further toxicity testing and residue data is similar to the Agency position regarding toxicity and the requirement of residue data for the microbial *Bt* products from which these plant-incorporated protectants were derived (see 40 CFR §§ 158.2130(d)(1)(i) and 158.2140(d)(7)). For microbial products, further toxicity testing and residue data are triggered by significant acute effects in studies, such as the mouse oral toxicity study, to verify and quantify the observed effects and clarify the source of these effects (Tiers II and III).

Cry3Bb1 protein residue chemistry data were not required for a human health effects assessment of the subject plant-incorporated protectant ingredients because of the lack of mammalian toxicity.

Neither available information concerning the dietary consumption patterns of consumers (and major identifiable subgroups of consumers including infants and children) nor safety factors that are generally recognized as appropriate for the use of animal experimentation data were evaluated. The lack of mammalian toxicity at high levels of exposure to the Cry3Bb1 proteins, as well as the minimal potential to be a food allergen, demonstrate the safety of the product at levels well above possible maximum exposure levels anticipated in the crop.

The genetic material necessary for the production of the plant-incorporated protectant active ingredients are the nucleic acids (deoxyribonucleic acid (DNA), ribonucleic acid (RNA)) that comprise genetic

material encoding these proteins and their regulatory regions. "Regulatory regions" are the genetic material—such as promoters, terminators, and enhancers—that control the expression of the genetic material encoding the proteins. DNA and RNA are common to all forms of plant and animal life, and the Agency knows of no instance where these nucleic acids have been associated with toxic effects related to their consumption as a component of food. These ubiquitous nucleic acids, as they appear in the subject active ingredients, have been adequately characterized by the applicant. Therefore, no mammalian toxicity is anticipated from dietary exposure to the genetic material necessary for the production of the subject active plant pesticidal ingredients. Further, the genetic material (DNA, RNA) necessary for the production of the Cry3Bb1 proteins have been exempted under the blanket exemption for all nucleic acids (40 CFR § 174.507).

ii. Infants and Children Risk Conclusions

FFDCA section 408(b)(2)(C) provides that EPA shall assess the available information about consumption patterns among infants and children, special susceptibility of infants and children to pesticide chemical residues, and the cumulative effects on infants and children of the residues and other substances with a common mechanism of toxicity.

In addition, FFDCA section 408(b)(2)(C) also provides that EPA shall apply an additional tenfold margin of safety for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of the data base unless EPA determines that a different margin of safety will be safe for infants and children.

In this instance, based on all the available information, the Agency concludes that there is a finding of no toxicity for the Cry3Bb1 proteins and the genetic material necessary for their production. Thus, there are no threshold effects of concern and, as a result, an additional margin of safety for infants and children is not necessary.

iii. Overall Safety Conclusion

There is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the Cry3Bb1 proteins and the genetic material necessary for their production. This includes all anticipated dietary exposures and all other exposures for which there is reliable information.

The Agency has arrived at this conclusion because, as discussed thus far in this chapter, no toxicity to mammals has been observed, nor has there been any indication of allergenicity potential for the plant-incorporated protectants.

e. Other Considerations

i. Analytical Enforcement Methodology

Methods for extraction and direct enzyme-linked immunosorbent assay (ELISA) analysis of Cry3Bb1 in corn grain have been submitted and found acceptable by the Agency.

ii. International Residue Limits

In making its tolerance decisions, EPA seeks to harmonize U.S. tolerances with international standards whenever possible, consistent with U.S. food safety standards and agricultural practices. In this context, EPA considers the international maximum residue limits (MRLs) established by the Codex Alimentarius Commission (Codex), as required by FFDCA section 408(b)(4). The Codex Alimentarius is a joint United Nations Food and Agriculture Organization/World Health Organization food standards program, and it is recognized as an international food safety standards-setting organization in trade agreements to which the United States is a party. EPA may establish a tolerance that is different from a Codex maximum residue level (MRL); however, FFDCA section 408(b)(4) requires that EPA explain the reasons for departing from the Codex level.

The Codex has not established a MRL for the Cry3Bb1 proteins in corn.

f. Endocrine Disruptors

As required under FFDCA section 408(p), the Agency has developed the Endocrine Disruptor Screening Program (EDSP) to determine whether certain substances (including pesticide active and other ingredients) may have an effect in humans or wildlife similar to an effect produced by a "naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." The EDSP employs a two-tiered approach to making the statutorily required determinations. Tier 1 consists of a battery of 11 screening assays to identify the potential of a chemical substance to interact with the estrogen, androgen, or thyroid (E, A, or T) hormonal systems. Chemicals that go through Tier 1 screening and are found to have the potential to interact with E, A, or T hormonal systems will proceed to the next stage of the EDSP where the Agency will determine which, if any, of the Tier 2 tests are necessary based on the available data. Tier 2 testing is designed to identify any adverse endocrine-related effects caused by the substance, and establish a quantitative relationship between the dose and the E, A, or T effect.

Between October 2009 and February 2010, the Agency issued test orders/data call-ins for the first group of 67 chemicals, which contains 58 pesticide active ingredients and 9 inert ingredients. This list of chemicals was selected based on the potential for human exposure through pathways such as food and water, residential activity, and certain post-application agricultural scenarios. This list should not be construed as a list of known or likely endocrine disruptors.

The Cry3Bb1 proteins are not among the group of 58 pesticide active ingredients on the initial list to be screened under the EDSP. Under FFDCA section 408(p), the Agency must screen all pesticide chemicals. Accordingly, the Agency anticipates issuing future EDSP orders/data call-ins for all pesticide active ingredients.

For further information on the status of the EDSP, the policies and procedures, the list of 67 chemicals, the test guidelines and the Tier 1 screening battery, please visit our website: *http://www.epa.gov/endo/*.

g. MON 863 90-Day Rat Feeding Study

The EPA and the Food and Drug Administration (FDA) did not request or receive the 90-day dietary study nor was there any formal review of the full study by either agency. Both agencies completed the safety evaluations for MON 863 corn and did not need the 90-day oral toxicity study to reach a finding on human dietary safety. However, other governments often request additional information or studies, and summary information on these studies are frequently provided to the U.S. governmental agencies on request. A summary of the study report was provided to the EPA.

The 90-day oral toxicity study (CV-2000-260) was performed by Convance Labs (Madison, Wisconsin) with high percentages of MON 863 corn (11 and 33%) used to make diet for the test rodents. The study was done according to Organization for Economic Cooperation and Development (OECD) guideline protocol 408 for a 90-day oral toxicity study and performed under the good laboratory practices guidelines. These testing requirements, routinely used for regulatory purposes, are actually more stringent in quality control procedures and record keeping than studies routinely reported in the scientific literature. An appropriate isoline corn control (a corn hybrid similar to MON 863 but not expressing the beetle control protein), and reference corn varieties (six different non-transgenic, commercial corn varieties) were employed to address the possible effects of different corn compositions on rat nutrition. The prepared diets were also analyzed to confirm that the proper test corn lines were used to make the animal feed. All test animals received diets specially prepared by Purina Mills formulated to contain 33% corn (conventional corn, MON 863 corn, or a combination) and provide a balanced diet.

It is important that a balanced diet be provided rather than feeding the test animals 100% corn so that any abnormalities that occur can be assigned to the effect of the corn component itself rather than any dietary insufficiency. It is also important that more than one level of MON 863 corn in the diet be included to establish if a dose-response is present. The dose-response shows that, if an effect is seen at a low dose, a more dramatic and similar effect will also occur at a higher dose. While dose-response is a key component to a toxicology assessment, it is difficult to establish in a whole food study. This is one of the reasons that EPA requests high dose purified protein toxicity studies for plant-incorporated protectants and compositional analysis is used for examining whole food.

The 90-day rat oral study with MON 863 was reviewed by both the Robert Koch Institute (RKI), as the German competent authority, and subsequently the European Food Safety Authority (EFSA). As could be anticipated by having such a large study with numerous treatments and comparisons, some

differences were found between treatments. In understanding the significance of these findings, it is important to be aware of the natural variation of biological systems in a population of test organisms and the range of responses to a treatment. As discussed in information provided by both RKI and EFSA, the decreases in male kidney weight and some changes in hematological parameters (lymphocyte and reticulocyte counts) for the highest MON 863-treatment groups were significantly different compared to the control isoline corn group. However, when compared to the included reference corn variety treatments, the values were found to be within the realm of normal biological variation. Since there were questions about possible kidney pathology, independent veterinary pathologists were asked to examine tissue samples and render an opinion. No significant findings relevant to an adverse toxicity determination were noted. Therefore, both RKI and EFSA found that there were no resultant concerns over the safety of MON 863 due to the results of the 90-day toxicity test.

Since the earlier examination of the study summaries of the 90-day feeding study for MON 863, a more recent publication by Séralini *et al*. (2007) has reanalyzed the data from the study. The paper states that there are significant effects due to MON 863 corn being present in the diet of the treated rodents. The authors postulate that new analyses of growth curves and blood chemistry parameters suggest significant hepatorenal effects. The Agency considers these new findings open to a different interpretation. The original studies did not demonstrate any changes in the blood chemistry parameters or body weight gains that showed dose-dependent responses. In addition, the organs most likely to be impacted by these chemistry changes (liver and kidney) were examined histologically in the original reports by independent, competent veterinary pathologists. The reports on these tissues indicated no signs of cellular toxicity were found in the hepatic or renal tissues. Without signs of cellular tissue damage in the relevant organs, the variations in blood chemistry should not be considered signs of toxicity.

h. MON 88017 Considerations (Reviewed in U.S. EPA (2005b))

The evaluation of mammalian toxicity of the Cry3Bb1 protein produced in MON 88017 is based on studies conducted with Cry3Bb1 protein variants (Cry3Bb1.11098 and Cry3Bb1.11231). The physicochemical characteristics of the Cry3Bb1 protein in MON 88017 were found to be similar with the Cry3Bb1 protein in MON 863. Moreover, the protein expression levels, insect bioactivity, and field efficacy data of Cry3Bb1 protein in MON 88017 were compared to MON 863 and found to be similar and functionally equivalent. Therefore, because the Cry3Bb1 proteins produced in MON 88017 and MON 863 share an amino acid sequence identity of >99.8% and the aforementioned similarities, the data (including the acute mouse gavage studies and *in vitro* digestibility studies previously submitted for MON 863) were bridged to support the finding that there is a reasonable certainty that no harm will result to from aggregate exposure to the U.S. population, including infants and children, to the Cry3Bb1 protein as expressed in MON 88017.

i Tolerance Exemption

Certain data submitted and reviewed for past experimental use permits, as well as the FIFRA section 3 registration of Corn Event MON 863 (EPA Reg. No. 524-528), also supported the petition for a

tolerance exemption for residues of the *Bt* Cry3Bb1 protein. Given the available data and information as summarized in Table 1, the Agency established a permanent exemption from the requirement of a tolerance for residues of the *Bt* Cry3Bb1 protein and the genetic material necessary for its production in corn when used as plant-incorporated protectants in the food and feed commodities of field corn, sweet corn, and pop corn under 40 CFR § 180.1214 (69 Federal Register (FR) 16809; March 31, 2004). Subsequently, administrative revisions were made to existing plant-incorporated protectant tolerance exemptions (e.g., some tolerance exemptions were moved from 40 CFR part 180 to 40 CFR part 174). The original Cry3Bb1 tolerance exemption was subject to these revisions as it was redesignated from 40 CFR § 180.1214 to 40 CFR § 174.518 and changed to the following (72 FR 20431; April 25, 2007):

> "**§ 174.518 *Bacillus thuringiensis* Cry3Bb1 protein in corn; exemption from the requirement of a tolerance.**
>
> Residues of *Bacillus thuringiensis* Cry3Bb1 protein in corn are exempt from the requirement of a tolerance when used as plant-incorporated protectants in the food and feed commodities of corn; corn, field; corn, sweet; and corn, pop."

Table 1. Human Health Assessment Data for Cry3Bb1 (Reviewed in U.S. EPA (2002a) Unless Otherwise Noted).

Study Title	Summary	MRID No.
Acute Oral Toxicity of *B.t.* Protein 11098 in Mice	There did not appear to be significant adverse effects to animals dosed with Cry3Bb1 at corrected dose amounts of 38.7, 419, or 2,980 milligrams per kilogram (mg/kg) bodyweight. Two animals died during the study—animal numbers 98035M3-007 and 98035F3-004 in the 2,980 mg/kg treatment group. These deaths appeared to be the result of trauma from dosing rather than from the test substance. In addition, although there were some minor weight loss and minor abnormal observations at gross necropsy, these occurred in both test and control groups and, therefore, do not appear to be Cry3Bb1.11098 protein exposure related. Based upon the data provided, the median lethal dose (LD_{50}) for Cry3Bb1.11098 is greater than 2,980 mg/kg bodyweight in mice. **Classification: Acceptable** **(Reviewed in U.S. EPA (2001a))**	449043-06
Acute Oral Toxicity Study of *B.t.* Protein 11231 in Mice	There were no apparent adverse effects identified in mice dosed orally with 36, 396 and 3,780 mg/kg bodyweight of Cry3Bb1.11231 protein. There was some minor weight loss in a few animals and some minor abnormal observations via gross necropsy, but these occurred in both the test and control groups and, therefore, do not appear to be Cry3Bb1.11231 protein exposure related. Based upon the data provided, the LD_{50} for Cry3Bb1.11231 is greater than 3,780 mg/kg bodyweight in mice. **Classification: Acceptable** **(Reviewed in U.S. EPA (2001b))**	449043-05

Study Title	Summary	MRID No.
An Acute Oral Toxicity Study in Mice with *E. coli* Produced Cry3Bb1.11098 (Q349R) Protein	There did not appear to be significant adverse affects to animals resulting from exposure to Cry3Bb1.11098 (Q349R) at dose amounts of 300, 900, and 2,700 mg/kg body weight. Observations included some minor clinical effects and a relatively insignificant lack of weight gain in two animals; however, these do not appear to be related to exposure to the test substance because these occurred in the various test groups. Based upon the data contained in this submission, the LD_{50} for Cry3Bb1.11098(Q349R) is greater than 3,200 mg/kg body weight in mice. **Classification: Acceptable**	455382-02
Assessment of the *in vitro* Digestibility of *B.t.* Protein 11098 and *B.t.* Protein 11231 Utilizing Mammalian Digestive Fate Models	The tests performed in this study show that the Cry3Bb1 proteins are not stable to digestion in simulated gastric fluid. Incubation of Cry3Bb1.11098 and Cry3Bb1.11231 in SGF results in the loss of detectable protein by the 30- and 15-second observation points, respectively, as detected by SDS-PAGE. Insect bioassay data indicated that the protein lost insecticidal activity within 2 minutes of incubation in SGF. Incubation in the simulated intestinal fluid (SIF) resulted in a ~59 kDa digestion product that retained its insecticidal activity for at least 30 minutes. **Classification: Acceptable** **(Reviewed in U.S. EPA (2001c))**	449043-07
Amended Report for MSL-15704: Assessment of the *in vitro* Digestibility of *B.t.* Protein 11098 and *B.t.* Protein 11231 Utilizing Mammalian Digestive Fate Models	The tests performed in this study show that the Cry3Bb1 proteins are not stable to digestion in simulated gastric fluid. Incubation of Cry3Bb1.11098 and Cry3Bb1.11231 in SGF results in the loss of detectable protein by the 30- and 15-second observation points, respectively, as detected by SDS-PAGE. Insect bioassay data indicated that the protein lost insecticidal activity within 2 minutes of incubation in SGF. Incubation in the SIF resulted in a ~59 kDa digestion product that retained its insecticidal activity after at least 30 minutes incubation. **Classification: Acceptable**	454240-06
Assessment of the *in vitro* Digestibility of Cry3Bb1 Protein Purified from Corn Event MON 863 and Cry3Bb1 Protein Purified from *E. coli*	The tests performed in this study show that the Cry3Bb1 proteins are degraded in simulated gastric fluid. Incubation of corn-produced and *Escherichia coli*-produced Cry3Bb1 protein in SGF results in the loss of detectable protein by the 15-second observation point, as detected by SDS-PAGE. **Classification: Acceptable**	455382-03

Study Title	Summary	MRID No.
Assessment of the *in vitro* Digestibility of the Cry3Bb1.11098 (Q349R) Protein in Simulated Intestinal Fluid	Simulated intestinal fluid activity was verified to be present and at a level deemed acceptable by SOP GEN-PRO-058-01. The gels provided indicate that the Cry3Bb1.11098 (Q349R) protein is present as a single band at 74 kDa, which rapidly degraded to two bands of 68 kDa and 57 kDa at the first assay time point of 1 minute. The subsequent samples (from 5 minutes to 24 hours) all gave a single 57 kDa band that did not appear to decrease in intensity. This lack of degradation by intestinal fluids is similar to the majority of Cry proteins, which are resistant to the action of trypsin. **Classification: Acceptable**	455770-02
Amended Report for MSL-16597: Immuno-Detectability of Cry3Bb1.11098 and Cry3Bb1.11231 Proteins in the Grain of Insect Protected Corn Events MON 863 and MON 853 After Heat Treatment	Heating the corn flour samples at 204°C for 30 minutes destroys both the immunoreactivity and insect bioactivity of the Cry3Bb1.11098 found in MON 863 corn. The Cry3Bb1 immunoreactivity was not detectable in both an immunoblot and ELISA format for MON 863. For MON 853, Cry3Bb1 was not recognizable in an immunoblot and reduced more than 1000-fold in an ELISA format. Since the rabbit anti-Cry3Bb1 antibody employed was polyclonal immunoglobulin G (IgG), it is also suggestive that epitopes were destroyed and not just rendered unrecognized by alteration of the three-dimensional configuration. **Classification: Acceptable**	454240-07
Bioinformatics Analysis of *B.t.* Protein 11098 and *B.t.* Protein 11231 Sequences Utilizing Toxin and Public Domain Genetic Databases	Several amino acid database comparison tools were employed to compare the amino acid sequence of Cry3Bb1.11098 and Cry3Bb1.11231 to known protein toxins. The TOXIN4 database was compiled to allow for comparison of Cry3Bb1.11098 and Cry3Bb1.11231 to these known toxin proteins. All of the protein similarities identified were to insecticidal protein with no similarity to proteins known to be toxic to humans and/or animals. Based upon these data, it does not appear that Cry3Bb1.11098 nor Cry3Bb1.11231 share significant structural, biological, or immunological similarity with known protein toxins other than those affecting insects. **Classification: Acceptable** **(Reviewed in U.S. EPA (2001d))**	449043-08

Study Title	Summary	MRID No.
Bioinformatics Analysis of *B.t.* Protein 11098 and *B.t.* Protein 11231 Sequences Utilizing an Allergen Database	Several amino acid database comparison tools were employed to compare the amino acid sequence of Cry3Bb1 to known protein allergens and gliadins. The UPDATE2 database was compiled to allow for comparison of Cry3Bb1.11098 and Cry3Bb1.11231 to these proteins. The level of similarity identified does not indicate significant similarity to any of the proteins or gliadins contained in the database. In addition, no contiguous stretch of 8 identical amino acids was identified in either the FASTA or IDENTITYSEARCH algorithms, suggesting a lack of immunological similarity. Based upon these data, it does not appear that Cry3Bb1 shares significant structural, biological, or immunological similarity with known protein allergens or gliadins. **Classification: Acceptable** **(MRID No. 449043-09 reviewed in U.S. EPA (2001e))**	449043-09 454240-08

Study Title	Summary	MRID No.
Safety Assessment of Cry3Bb1 Variants in Corn Rootworm Protected Corn	Plants transformed for corn rootworm control (Event MON 863) contained a total of seven amino acid changes within the Cry3Bb1.11098 delta-endotoxin when compared to the sequence as found in wild type *Bt. Bt* strains EG11231 and EG11098 contain variants of the Cry3Bb1 protein, which differ from the wild type delta-endotoxin by 4 and 5 amino acids, respectively. Two further alterations in amino acid sequence were made for Cry3Bb1.11098 during cloning and insertion into the maize genome. Structural data indicate that these alleles of this protein maintained a very similar structure to the native form. The initial transformation event used to evaluate the rootworm-protected maize was MON 853, which encodes Cry3Bb1 variant 11231. Protein produced by fermentation of *Bt* cells expressing variant 11231 was used in toxicology studies for environmental and mammalian concerns. Functional and physicochemical equivalence between variant 11231 produced in *Bt* and that produced in MON 853 were demonstrated. Maize was also transformed with variant 11098, resulting in transformation Event MON 863. These two variants, 11098 and 11231, were shown to be physicochemically and functionally equivalent. The registrant stated that an examination of toxicity toward catfish, bobwhite quail, *Daphnia magna*, Collembola (*Folsomia candida*), adult and larval honeybees, a ladybird beetle, a green lacewing, a parasitic wasp, and earthworms resulted in a NOEC (No Observable Effect Concentration) being established that exceeded the concentration of Cry3Bb1 toxins expected in the maximum environmental exposure. No Observable Effect Concentrations surpassed maximum predicted environmental concentrations by 3 to 141 fold, hence, the risk to non-target organisms from the culture of MON 863 is indicated to be minimal. However, this aspect is the subject of another review and outside the purview of this report. Given the lack of known mechanisms of mammalian toxicity from *Bt* delta-endotoxins, their widespread use in agriculture, the rapid digestibility of Cry3Bb1 proteins, their lack of homology to known toxins and allergens, and the safety of the microbial biopesticide Raven®, which expresses Cry3Bb1 proteins, the Cry3Bb1 protein is expected to have a reasonable certainty of causing no harm in its aggregate exposure. **Classification: Acceptable** **(Reviewed in U.S. EPA (2002b))**	454240-09

Study Title	Summary	MRID No.
Human Health and Environmental Assessment of the Plant-Incorporated Protectant *Bacillus thuringiensis* Cry3Bb1 Protein Produced in MON 88017	This report is a summary of the product characterization studies and the human health assessment for the plant-incorporated protectant, *Bt* Cry3Bb1 protein produced in MON 88017. All physiochemical characteristics in the Cry3Bb1 protein in MON 88017 were found to be similar to the Cry3Bb1 protein in MON 863. Moreover, the protein expression levels, insect bioactivity, and field efficacy data of Cry3Bb1 protein in MON 88017 were compared to MON 863 and found to be similar and functionally equivalent. Based on substantial similarity of Cry3Bb1 protein in MON 88017 and the Cry3Bb1 protein produced in the registered MON 863 corn product, data were bridged from the studies previously submitted for MON 863. Collectively, these studies demonstrated that the Cry3Bb1 protein variants are not toxic to mammals, are rapidly and completely digested in simulated gastric fluid, do not share any amino acid homology to known toxins or allergens, and are unlikely to produce a toxic or allergic response in humans. Therefore, there is a reasonable certainty that no harm will result from potential exposure to the Cry3Bb1 protein in transgenic corn MON 88017. **Classification: Acceptable** **(Reviewed in U.S. EPA (2005a))**	461817-01

3. Human Health Assessment of Cry1Ab (Expressed in MON 863 x MON 810 and MON 88017 x MON 810), NPTII (Expressed in Corn Event MON 863 and MON 863 x MON 810), and CP4 EPSPS (Expressed in MON 88017 and MON 88017 x MON 810)

Based on previously completed Agency human health assessments, permanent tolerance exemptions have been established for the Cry1Ab, neomycin phosphotransferase II (NPTII), and CP4 5-enolpyruvylshikimate-3-phosphate (CP4 EPSPS) proteins:

> "**§ 174.511 *Bacillus thuringiensis* Cry1Ab protein in all plants; exemption from the requirement of a tolerance.**
>
> Residues of *Bacillus thuringiensis* Cry1Ab protein in all plants are exempt from the requirement of a tolerance when used as plant-incorporated protectants in all food commodities."
>
> **original under 40 CFR § 180.1173 (61 FR 40340; August 2, 1996)
> **revised under 40 CFR § 174.511 (72 FR 20431; April 25, 2007)

"**§ 174.521 Neomycin phosphotransferase II; exemption from the requirement of a tolerance.**

Residues of the neomycin phosphotransferase II (NPTII) enzyme are exempted from the requirement of a tolerance in all food commodities when used as a plant-incorporated protectant inert ingredient."

**original under 40 CFR § 180.1134 (59 FR 49351; September 28, 1994)
**revised under 40 CFR § 174.521 (72 FR 20431; April 25, 2007)

"**§ 174.523 CP4 Enolpyruvylshikimate-3-phosphate (CP4 EPSPS) synthase in all plants; exemption from the requirement of a tolerance.**

Residues of the CP4 Enolpyruvylshikimate-3-phosphate (CP4 EPSPS) synthase enzyme in all plants are exempt from the requirement of a tolerance when used as plant-incorporated protectant inert ingredients in all food commodities."

**original under 40 CFR § 180.1174 (61 FR 40338; August 2, 1996)
**revised under 40 CFR § 174.523 (72 FR 20431; April 25, 2007)

The toxicological and allergenicity data supporting the establishment of the Cry1Ab tolerance exemption, as well as the associated registrations of MON 863 x MON 810 and MON 88017 x MON 810, can be found in the 2001 *Bt* Crops Reassessment and/or the September 2010 Cry1Ab and Cry1F BRAD (U.S. EPA 2001f and 2010).

The individual data generated for the Cry1Ab, NPTII, and CP4 EPSPS proteins (and Cry3Bb1) support their inclusion, as expressed in particular stacked plant-incorporated protectants (i.e., MON 863 x MON 810 and MON 88017 x MON 810), into the appropriate tolerance exemptions since the mode of action for these proteins does not suggest a synergistic activity in combination for mammalian species (U.S. EPA 2003, 2005b). This lack of synergism is also suggested by the absence of enhanced responses in sensitive target species tested with the combination of Cry1Ab and Cry3Bb1 proteins.

Human health assessment data, provided specifically in relation to the Corn Event MON 863 registration, are summarized in Table 2.

Table 2. Human Health Assessment Data for NPTII (Reviewed in U.S. EPA (2002a)).

Study Title	Summary	MRID No.
Immuno-Detectability of NPTII Protein in the Grain of Insect Protected Corn Event MON 863 After Heat Treatment	The immunoblot shows that extraction of the MON863 corn grain spiked with NPTII yielded an immunoreactive band that comigrated with the *E. coli*-produced NPTII. The blot also showed that, regardless of the extraction buffer used, the heat treatment effectively removed any immunoreactive bands from the samples. The results suggest that, even if detectable levels of NPTII were present in MON863 corn grain, the heat treatment would remove them. Unfortunately, the use of a mouse monoclonal antibody limits the ability of this data to be extrapolated. A heat treatment, significantly above the 95.8°C used for sample preparation for SDS-PAGE, destroyed the epitope(s) recognized by the anti-NPTII antibody used. **Classification: Acceptable**	455382-09

4. References

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MRID No. 449043-06. Bechtel C. 1999. Acute Oral Toxicity of *B.t.* Protein 11098 in Mice. Lab Project Number: 16215: ML-98-141: MSE-N 98035. Unpublished study prepared by Monsanto Company, 212 pages.

MRID No. 449043-07. Leach J, Hileman R, Martin J. 1999. Assessment of the *in vitro* Digestibility of *B.t.* Protein 11098 and *B.t.* Protein 11231 Utilizing Mammalian Digestive Fate Models. Lab Project Number: 98-01-39-02: 15704. Unpublished study prepared by Monsanto Company, 40 pages.

MRID No. 449043-08. Astwood J, Hileman R. 1999. Bioinformatics Analysis of *B.t.* Protein 11098 and *B.t.* Protein 11231 Sequences Utilizing Toxin and Public Domain Genetic Databases. Lab Project Number: 15870. Unpublished study prepared by Monsanto Company, 294 pages.

MRID No. 449043-09. Astwood J, Hileman R. 1999. Bioinformatics Analysis of *B.t.* Protein 11098 and *B.t.* Protein 11231 Sequences Utilizing an Allergen Database. Lab Project Number: 15873. Unpublished study prepared by Monsanto Company, 38 pages.

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MRID No. 454240-09. Hileman R, Astwood J, McKee M. 2001. Safety Assessment of Cry3Bb1 Variants in Corn Rootworm Protected Corn. Lab Project Number: 17225: MSL-17225. Unpublished study prepared by Monsanto Company, 51 pages.

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U.S. EPA. 2001b. Data Evaluation Report on MRID No. 449043-05 ("Acute Oral Toxicity Study of *B.t.* Protein 11231 in Mice"). Completed by M.T. Watson, Ph.D. and J. L. Kough, Ph.D. on April 25, 2001.

U.S. EPA. 2001c. Data Evaluation Report on MRID No. 449043-07 ("Assessment of the *in vitro* Digestibility of *B.t.* Protein 11098 and *B.t.* Protein 11231 Utilizing Mammalian Digestive Fate"). Completed by M.T. Watson, Ph.D. and J.L. Kough, Ph.D. on April 25, 2001.

U.S. EPA. 2001d. Data Evaluation Report on MRID No. 449043-08 ("Bioinformatics Analysis of *B.t.* Protein 11098 and *B.t.* Protein 11231 Sequences Utilizing Toxin and Public Domain Genetic Databases"). Completed by M.T. Watson, Ph.D. and J.L. Kough, Ph.D. on April 25, 2001.

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C. Environmental Assessment

1. Tiered Hazard and Risk Assessment Process

To minimize data requirements and avoid unnecessary tests, risk assessments are structured such that risk is determined first from estimates of hazard under "worst-case" exposure conditions. A lack of adverse effects under these conditions would provide enough confidence that there is no risk and no further data would be needed. Hence, such screening tests conducted early in an investigation tend to be broad in scope, but relatively simple in design, and can be used to demonstrate acceptable risk under most conceivable conditions. When screening studies suggest potentially unacceptable risk, additional studies are designed to assess risk under more realistic field exposure conditions. These later tests are more complex than earlier screening studies. Use of this "tiered" testing framework saves valuable time and resources by organizing the studies in a cohesive and coherent manner and eliminating unnecessary lines of investigation. Lower tier, high-dose screening studies also allow tighter control over experimental variables and exposure conditions, resulting in a greater ability to produce statistically reliable results at relatively low cost.[a]

Tiered tests are designed to first represent unrealistic worst-case scenarios and ONLY progress to real-world field scenarios if the earlier tiered tests fail to indicate adequate certainty of acceptable risk. Screening (Tier I) non-target organism hazard tests are conducted at exposure concentrations several times higher than the highest concentrations expected to occur under realistic field exposure scenarios. This has allowed an endpoint of 50% mortality to be used as a trigger for additional higher tier testing. Less than 50% mortality under these conditions of extreme exposure suggest that population effects are likely to be negligible given realistic field exposure scenarios.

The Environmental Protection Agency (EPA) uses a tiered (Tiers I–IV) testing system to assess the toxicity of a plant-incorporated protectant (PIP) to representative non-target organisms that could be exposed to the toxin in the field environment. Tier I high-dose studies reflect a screening approach to testing designed to maximize any toxic effects of the test substance on the test (non-target) organism. The screening tests evaluate single species in a laboratory setting with mortality as the endpoint. Tiers II–IV generally encompass definitive hazard level determinations, longer term greenhouse or field testing, and are implemented when unacceptable effects are seen at the Tier I screening level.

Testing methods, which utilize the tiered approach, were last published by EPA as Harmonized Office of Prevention, Pesticides, and Toxic Substances (OPPTS) Testing Guidelines (now Harmonized Office

[a] Non-target invertebrate hazard tests often are conducted at exposure concentrations several times higher than the maximum concentrations expected to occur under realistic exposure scenarios. This has customarily allowed an endpoint of 50% mortality to be used as a trigger for additional higher tier testing. Lower levels of mortality under these conditions of extreme exposure suggest that population effects are likely to be negligible given realistic exposure scenarios. Thus, it follows that the observed proportion of responding individuals can be compared to a 50% effect to determine if the observed proportion is significantly lower than 50%. For example, using a binomial approach, a sample size of 30 individuals is sufficient to allow a treatment effect of 30% to be differentiated from a 50% effect with 95% confidence using a one-sided Z test. A one-sided test is appropriate because only effects of less than 50% indicate that further experiments are not needed to evaluate risk.

of Chemical Safety and Pollution Prevention (OCSPP) Testing Guidelines), Series 850 and 885 (EPA 712-C-96-280, February 1996).[b] These guidelines apply to microbes and microbial toxins when used as pesticides, including those that are naturally occurring and those that are strain improved either by natural selection or by deliberate genetic manipulation. Therefore, plant-incorporated protectants (PIPs) containing microbial toxins are also covered by these testing guidelines.

The Tier I screening maximum hazard dose (MHD) approach to environmental hazard assessment is based on some factor (whenever possible >10) times the maximum amount of active ingredient expected to be available to terrestrial and aquatic non-target organisms in the environment, or the Estimated Environmental Concentration (EEC).[c] Tier I tests serve to identify potential hazards and are conducted in the laboratory at high dose levels, which increase the statistical power to test the hypotheses. Elevated test doses, therefore, add certainty to the assessment, and such tests can be well standardized. The Guidelines call for initial screening testing of a single group or several groups of test animals at the maximum hazard dose level. The Guidelines call for testing of one treatment group of at least thirty animals or three groups of ten test animals at the screening test concentration. The Guidelines further state that the duration of all Tier I tests should be approximately 30 days. Some test species, notably non-target insects, may be difficult to culture and the suggested test duration has been adjusted accordingly. Control and treated insects should be observed for at least 30 days, or in cases where an insect species cannot be cultured for 30 days, until negative control mortality rises above 20%.

Failing the Tier I (10x EEC) screening at the MHD does not necessarily indicate the presence of an unacceptable risk in the field, but it triggers the need for additional testing.[d] A less than 50% mortality effect at the MHD is taken to indicate minimal risk. Greater than 50% mortality, however, does not necessarily indicate the existence of unacceptable risk in the field, but it does trigger the need to collect additional dose-response information and a refinement of the exposure estimation before deciding if the risk is acceptable or unacceptable. Where potential hazards are detected in Tier I testing (i.e., mortality is greater than 50%), additional information at lower test doses is required, which can serve to confirm whether any effect might still be detected at more realistic field (1x EEC) concentrations and routes of exposure.[e]

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[b] General OCSPP Harmonized Testing Guidelines available from: *http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm*.
Series 850 Testing Guidelines available from:
http://www.epa.gov/ocspp/pubs/frs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/index.html.
Series 885 Testing Guidelines available from:
http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series885.htm.

[c] The dose margin can be less than 10x where uncertainty in the system is low or where high concentrations of test material are not possible to achieve due to test organism feeding habits or other factors. High-dose testing also may not be necessary where many species are tested or tests are very sensitive, although the test concentration used must exceed 1x EEC.

[d] Note that the 10x EEC MHD testing approach is not equivalent to what is commonly known as "testing at a 10x safety factor," where any adverse effect is considered significant. Tier I screen testing is not "safety factor testing." In a "10x safety factor" test, any adverse effect noted is a "level of concern," whereas in the EPA environmental risk assessment scenario any adverse effect is viewed as a concern only at 1x the field exposure.

[e] The 1x EEC test dose is based on plant tissue content and is considered the highest dose in a worst-case scenario (sometimes referred to as the Highest Estimated Environmental Concentration or HEEC). This 1x EEC is still much greater than any amount which any given non-target organism may be ingesting in the field because most non-target organisms do

When screening tests indicate a need for additional data, the OCSPP Harmonized Guidelines call for testing at incrementally lower doses in order to establish a definitive LD_{50} (i.e., dose that will kill 50% of the test organisms within a designated period) and to quantify the hazard. In the definitive testing, the number of doses and test organisms evaluated must be sufficient to determine an LD_{50} value and, when necessary, the Lowest Observed Adverse Effect Concentration (LOAEC), No Observed Adverse Effect Level (NOAEL), or reproductive and behavioral effects such as feeding inhibition, weight loss, etc. In the final analysis, a risk assessment is made by comparing the LOAEC to the EEC; when the EEC is lower than the LOAEC, a no risk conclusion is made. These tests offer greater environmental realism, but they may have lower statistical power. Appropriate statistical methods, and appropriate statistical power, must be employed to evaluate the data from the definitive tests. Higher levels of replication, test species numbers, and/or repetition are needed to enhance statistical power in these circumstances.

Data that shows less than 50% mortality at the maximum hazard dosage level (i.e., LC_{50}, ED_{50}, or LD_{50} >10x EEC) is sufficient to evaluate adverse effects, making lower field exposure dose definitive testing unnecessary. It is also notable that the recommended >10x EEC maximum hazard dose level is a highly conservative factor. The published EPA Level of Concern (LOC) is 50% mortality at 5x EEC (U.S. EPA 1998).[f]

Validation: The tiered hazard assessment approach was developed for EPA by the American Institute of Biological Sciences (AIBS) and confirmed in 1996 as an acceptable method of environmental hazard assessment by a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) on microbial pesticides and microbial toxins. The December 9, 1999 SAP agreed that the tiered approach was suitable for use with PIPs; however, this panel recommended that, for PIPs with insecticidal properties, additional testing of beneficial invertebrates closely related to target species and/or likely to be present in genetically modified (GM) crop fields should be conducted. Testing of *Bacillus thuringiensis* (*Bt*) Cry proteins on species not closely related to the target insect pest was not recommended, although it is still performed to fulfill the published EPA non-target species data requirements. In October 2000, another SAP also recommended that field testing should be used to evaluate population-level effects on non-target organisms. The August 2002 SAP, and some public comments, generally agreed with this approach, with the additional recommendation that indicator organisms should be selected on the basis of potential for field exposure to the subject protein (U.S. EPA 2000a, 2001a, 2002f, and 2004a).

Chronic studies: Since delayed adverse effects and/or accumulation of toxins through the food chain are not expected to result from exposure to proteins, protein toxins are not routinely tested for chronic effects on non-target organisms. But, the 30-day test duration requirement does amount to subchronic testing when performed at field exposure test doses. Proteins do not bioaccumulate. The biological

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not ingest plant tissue.

[f] The established peer and EPA Science Board reviewed guidance on screening test levels of concern is 50% mortality at 5x environmental concentration for terrestrial and 10x for aquatic species. The appropriate endpoints in high-dose limit/screening testing are based on mortality of the treated, as compared to the untreated (control) non-target organisms. A single group of 30 test animals may be tested at the maximum hazard dose.

nature of proteins makes them readily susceptible to metabolic, microbial, and abiotic degradation once they are ingested or excreted into the environment. Although there are reports that some proteins (Cry proteins) bind to soil particles, it has also been shown that these proteins are degraded rapidly by soil microbial flora upon elution from soil particles.

Conclusion: The tiered approach to test guidelines ensures, to the greatest extent possible, that the Agency requires the minimum amount of data needed to make scientifically sound regulatory decisions. EPA believes that maximum hazard dose Tier I screening testing presents a reasonable approach for evaluating hazards related to the use of biological pesticides and for identifying negative results with a high degree of confidence. The Agency expects that Tier I testing for short-term hazard assessment will be sufficient for most studies submitted in support of PIP registrations. If long-range adverse effects must be ascertained, however, then higher tier, longer term field testing will be required. As noted above, the October 2000 SAP and the National Academy of Sciences (NAS 2000) recommended testing non-target organisms directly in the field. This approach, with an emphasis on testing invertebrates found in corn fields, was also recommended by the August 2002 SAP and was supported by several public comments. Based on these recommendations and due to the lack of baseline data on the potential for long-term environmental effects from the cultivation of PIP-producing plants, the Agency has required long-term field studies on invertebrate populations/communities and Cry protein accumulation in soils as conditions of past PIP registrations.

Since the commercialization of *Bt* crops, the number of field studies published in scientific literature, in combination with the post-registration field studies submitted to the Agency, has accumulated to a level where empirical conclusions can be made. As a result, the issue of long-range effects of cultivation of these Cry proteins on the invertebrate community structure in *Bt* crop fields has since been adequately addressed. Specifically, a meta-analysis[g] of the data collected from 42 field studies indicated that non-target invertebrates are generally more abundant in *Bt* cotton and *Bt* maize fields than in non-transgenic fields managed with insecticides (Marvier *et al*. 2007). In addition, a comprehensive review of short- and long-term field studies on the effects of invertebrate populations in *Bt* corn and cotton fields indicated that no unreasonable adverse effects are taking place as a result of wide-scale *Bt* crop cultivation (Sanvido *et al*. 2007). Another review of field tests published to date concluded that the large-scale studies in commercial *Bt* cotton have not revealed any unexpected non-target effects other than subtle shifts in the arthropod community caused by the effective control of the target pests (Romeis *et al*. 2006). Slight reductions in some invertebrate predator populations are an inevitable result of all pest management practices, which result in reductions in the abundance of the pests as prey.

Overall, the Agency is in agreement with the conclusions of these studies and, collectively, these results provide extensive data to support that *Bt* crops have not caused long-term environmental effects, on a population level, to organisms not targeted by *Bt* proteins. Based on these considerations, regulatory

[g] This research was funded by EPA grant CR-832147-01. The *Bt* crop non-target effects database can be found on the National Center for Ecological Analysis and Synthesis (NCEAS) Web Site: *http://delphi.nceas.ucsb.edu/btcrops/*.

testing of the specialist predators and parasitoids of target pests may eventually be considered unnecessary.

2. Corn Event MON 863 (Organization for Economic Cooperation and Development (OECD) Unique Identifier: MON-ØØ863-5) Expressing Cry3Bb1

a. Data Cited/Submitted for Initial Registration of Corn Event MON 863 (Prior to February 2003)

i. Background

In 2000, Monsanto Company ("Monsanto") requested registration for *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector PV-ZMIR13L) in MON 863 corn (OECD Unique Identifier: MON-ØØ863-5). This protein controls the corn rootworm (CRW, *Diabrotica* spp.), a primary pest of corn in the United States. Corn rootworm larvae feed on corn roots, resulting in lodging and a reduction in a plant's ability to absorb water and nutrients from soil. In areas where the CRW is a pest (e.g., Corn Belt), significant financial losses are realized from decreased corn yields and increased expenditures on chemical pest control agents, including organophosphate, carbamate, and pyrethroid insecticides.

EPA conducted an environmental hazard assessment of the Cry3Bb1-producing corn lines. The general topics covered include gene flow to related wild plants, development of weediness, effects on wildlife, and fate of Cry3Bb1 in the environment. The assessment is based on data submitted to the Agency during the development of the corn lines; additional data submitted for registration; FIFRA SAP recommendations; consultations with scientific experts; and previous public comments on plant-incorporated protectant regulation.

ii. Non-Target Wildlife Hazard Assessment

Two separate SAP reports (October 2000 and August 2002) recommended that non-target testing should focus on species exposed to the crop being registered. In addition to testing species directly exposed to the CryBb1 protein in the field, however, the full battery of non-target wildlife species testing was conducted to comply with the published Agency non-target data requirements for microbial toxins. The Agency has determined that the non-target organisms most likely to be exposed to the protein in transgenic corn fields are beneficial insects feeding on corn pollen and nectar, and soil invertebrates, particularly coleopteran species. Initially, in lieu of extensive and difficult laboratory soil coleopteran toxicity testing followed by an extrapolation to community risk assessment, direct field census data and data on coleopteran insect effects and abundance in the field were requested, received, and evaluated. The August 2002 SAP, however, found the field census data unsatisfactory because of low statistical power. Therefore, maximum hazard dose toxicity testing on representative beneficials from several taxa was performed. The toxicity of the Cry3Bb1 protein has been evaluated on several species of invertebrates, including adult and larval honey bees, a parasitic hymenopteran (*Nasonia vitripennis*),

green lacewings, lady beetles, collembola, monarch butterfly, and earthworms. Reproductive and developmental observations were also made on collembola, honey bee, and lady beetle larva. The August 2002 SAP (as well as several public comments), however, found the green lacewing and parasitic wasp studies lacking and recommended testing of alternative species. Lastly, based on worst-case soil concentration, soil degradation studies show that Cry3Bb1 protein in corn tissue is no longer detectable in agricultural field soil after 22 to 28 days. The August 2002 SAP (as well as several public comments), however, suggested that additional soil degradation testing is desirable in a larger variety of soils and climatic conditions.

The non-target organisms tested are chosen as representative indicators of the major groups of wildlife and on the potential for field exposure as deduced from data on Cry3Bb1 protein expression in the plant. Although *Bt* Cry proteins are very specific in their activity to only certain insect species, for Cry3Bb1 protein in corn, the Agency has examined the toxicity to birds, fish, honey bees, and certain other beneficial insects even though the October 2000 SAP recommended against testing of non-target species not related to those susceptible to the specific activity of *Bt* Cry proteins. Nevertheless, in order to comply with the Agency's published data requirements for registration of microbial toxins (40 CFR § 158.2150), the Agency asked for avian and aquatic invertebrate toxicity data, as well as data on collembola (springtail) and earthworm species to ascertain effects on beneficial soil invertebrates because prolonged exposure to Cry3Bb1 protein in soil was a possibility. Earthworm studies were also conducted and submitted to demonstrate a lack of Corn Event MON 863 effects on beneficial decomposers. Honey bee effects on brood, as well as adults, were also required as exposure of honey bees to the Cry3Bb1 protein in pollen is expected.

The form of the test substances used in the studies for this assessment are plant material such as leaves, roots, pollen, or purified, bacterially produced Cry3Bb1 protein incorporated into the test species diet. The October 2000 SAP provided guidance to the Agency that, while actual plant material is the preferred test material, bacterially derived protein is also a valid test substance, particularly in testing where the test animals do not consume corn plant tissue and where large amounts of Cry protein are needed for maximum hazard dose testing. As per the OCSPP Harmonized Testing Guidelines, the adult insect studies were generally of 30 days duration or until the negative control mortality reached 20%. Larval studies were carried out through pupation and adult emergence.

The results for each study on environmental effects for Cry3Bb1 are summarized in Table 1. Additionally, the results are presented in a more descriptive format in subsequent sections of this Environmental Assessment chapter. Complete reviews of each study can be found in the individual Data Evaluation Reports.

Table 1. Environmental Effects Data for Corn Event MON 863.

Guideline Number	Study	Results	MRID Number
885.4050	Avian Dietary Testing	The dietary median lethal concentration (LC_{50}) value for Cry3Bb1 corn grain to juvenile northern bobwhite was greater than 70,000 parts per million (ppm) (10% of the diet) in a 8-day study (8-day observation). No adverse effects on avian wildlife are expected from incidental field exposure to Cry3Bb1 corn. A higher corn concentration and longer duration broiler study with MON863 corn is recommended. **Classification: Supplemental** **(Reviewed in U.S. EPA (2003a and 2003g)** *Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.	449043-15
885.4150	Wild Mammal Testing	Mammalian wildlife exposure to Cry3Bb1 protein is considered likely; however, the Cry3Bb1 toxicity data, as described in the Human Health Assessment (see section II(B)(2) of this Biopesticides Registration Action Document (BRAD)), indicate that there is no significant toxicity to rodents from testing at the maximum hazard dose. Therefore, no hazard to mammalian wildlife is anticipated, and data on wild mammal testing are not required. **(Reviewed in U.S. EPA (2003g))**	N/A
885.4200	Freshwater Fish Testing	In an 8-week subchronic study, no treatment mortality or behavior change was observed among channel catfish when fed diets containing 35% Cry3Bb1 corn lines MON 853 and MON 859. **Classification: Acceptable** **(Reviewed in U.S. EPA (2003b and 2003g))**	449043-19
885.4200	Freshwater Fish Testing	The requirement for a freshwater fish (rainbow trout) static-renewal toxicity study has been waived based on a lack of any substantial exposure of fish to the Cry3Bb1 proteins produced in corn crops. **(Reviewed in U.S. EPA (2003g))**	N/A

Guideline Number	Study	Results	MRID Number
850.1010	Aquatic Invertebrate Acute Toxicity Test (*Daphnia magna*)	The 48-hour LC_{50} value for Cry3Bb1 corn pollen, when administered to neonate daphnids, was >120 milligrams (mg) pollen/liter (L), a maximum hazard dose. No other adverse effects were noted. Therefore, no hazards to daphnia are expected from incidental exposure to Cry3Bb1-containing corn pollen. **Classification: Acceptable** **(Reviewed in U.S. EPA (2003c and 2003g))** *Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.	449043-18
885.4280	Estuarine and Marine Animal Testing	The estuarine and marine animal studies are waived for this product because of very low to no potential for exposure to Cry3Bb1 protein from field corn. **(Reviewed in U.S. EPA (2003g))**	N/A
885.4300	Non-Target Plant Studies	Since the active ingredient in this product is an insect toxin (*Bt* endotoxin) that has never shown any toxicity to aquatic or terrestrial plants, these studies have been waived for this product. Outcrossing issues in section II(C)(2)(a)(ii)(V) of this BRAD. **(Reviewed in U.S. EPA (2003g))**	N/A
885.4380	Honey Bee Larva Testing	The LC_{50} for honey bee larvae and maturation to adult bees was determined to be >1,790 ppm Cry3Bb1 protein (100x the concentration in pollen) in a maximum hazard dose study. Therefore, no hazard to honey bee larvae and adult bee emergence is anticipated. **Classification: Acceptable** **(Reviewed in U.S. EPA (2002a, 2002e, 2003f, and 2003g))**	449043-10
885.4380	Adult Honey Bee Testing	An adult honey bee maximum hazard dose feeding study showed the LC_{50} of the Cry3Bb1 protein to be >360 micrograms per milliliter (µg/mL) (20x the concentration found in pollen). Therefore, no hazard from the Cry3Bb1 protein to honey bees is expected. **Classification: Acceptable** **(Reviewed in U.S. EPA (2002a, 2002e, 2003f, and 2003g))**	449043-11

Guideline Number	Study	Results	MRID Number
885.4340	Parasitic Hymenoptera Larva Testing	The LC_{50} for parasitic Hymenoptera was determined to be >8,000 ppm Cry3Bb1 protein. Parasitic Hymenoptera are not expected to feed directly on corn plant tissue. Therefore, minimal exposure and no hazard to parasitic Hymenoptera from Cry3Bb1 protein are expected. Testing of a species more common to corn fields is recommended. **Classification: Acceptable** **(Reviewed in U.S. EPA (2002a, 2002e, 2003f, and 2003g))** *Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.	449043-13
885.4340	Dietary Toxicity to Green Lacewing Larvae	The LC_{50} for green lacewing larvae was determined to be >8,000 ppm Cry3Bb1 protein (20x field exposure). Based on these results, it can be concluded that green lacewing will not be adversely affected when exposed to Cry3Bb1 in the field. Because of questionable ingestion of the test material, another species (e.g., minute pirate bug or predatory carabid), more likely to be exposed to Cry3Bb1, should be tested. **Classification: Acceptable** **(Reviewed in U.S. EPA (2002a, 2002e, 2003d, and 2003g))** *Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.	449043-12
885.4340	Effects of *Bt* Protein 11231 on Adult Lady Beetles (*Hippodamia convergens*)	This maximum hazard dose study showed that the LC_{50} for Cry3Bb1, when fed to adult *H. convergens* is >8,000 micrograms (µg) purified *Bt* protein/milliliter (mL) diet, equivalent to 20x the maximum *Bt* protein concentration in plant tissue. A follow-up pollen feeding study was requested. **Classification: Acceptable** **(Reviewed in U.S. EPA (2002e and 2003g))**	449043-14
885.4340	Lady Beetle Larval Feeding Study (*Coleomegilla maculata*)	The LC_{50} for Cry3Bb1 expressed in pollen is >93 micrograms per gram (µg/g) fresh pollen weight. The larvae were observed through pupation to adult emergence. It can be concluded from this study that *Coleomegilla maculata* larvae will not be adversely affected by Cry3Bb1 field corn pollen. **Classification: Acceptable** **(Reviewed in U.S. EPA (2002a, 2002e, and 2003g))**	455382-04

Guideline Number	Study	Results	MRID Number
885.4340	Adult Lady Beetle Pollen Feeding Study (*Coleomegilla maculata*)	No significant adverse effects were noted in a 30-day 50% pollen feeding study. Based on these results, no hazard to *C. maculata* is expected when feeding on Cry3Bb1 corn pollen in the field. **Classification: Acceptable** **(Reviewed in U.S. EPA (2002d, 2002e, and 2003g))**	453613-01
885.4340	Adult Lady Beetle Pollen Feeding Study (*Hippodamia convergens*)	No significant adverse effects were noted in a 15-day 50% pollen in honey water feeding study. Based on these results, no hazard to *H. convergens* is expected if feeding on Cry3Bb1corn pollen in the field. **Classification: Acceptable** **(Reviewed in U.S. EPA (2002d, 2002e, and 2003g))**	453613-02
885.4340	Collembola Chronic Dietary Toxicity Study	The LC_{50} of the Cry3Bb1 protein for collembola was found to be >872.5 µg (50% corn leaf tissue in the diet). No adverse reproductive effects were noted. It can be concluded from this test that Cry3Bb1protein does not pose a hazard to collembola, a representative of a beneficial decomposer soil-inhabiting species. **Classification: Acceptable** **(Reviewed in U.S. EPA (2002a, 2002e, and 2003g))**	449043-17
850.6200	Earthworm Toxicity Study	A maximum hazard dose 14-day LC_{50} for earthworms exposed to Cry3Bb1 protein in an artificial soil substrate was determined to be >570 mg Cry3Bb1 protein/kilogram (kg) dry soil, or greater than 10x the maximum EEC of the protein. The data show that no adverse effects to earthworms are expected from exposure to corn plants producing Cry3Bb1 protein. **Classification: Supplemental** **(Reviewed in U.S. EPA (2002e, 2003e, and 2003g))**	449043-16
N/A	Earthworm Toxicity Study	There were no earthworm mortalities or other remarkable observations during the 14-day study. The LC_{50} value is greater than the highest maximum hazard concentration tested (166.6 mg Cry3Bb1 protein variant 11098 (Q349R)/kg dry soil). **Classification: Acceptable** **(Reviewed in U.S. EPA (2003f and 2003g))**	457571-01

Guideline Number	Study	Results	MRID Number
885.4340	Monarch Butterfly Larval Pollen Feeding Study	This study demonstrated that corn pollen expressing the Cry3Bb1 protein will not result in acute toxic or developmental effects to monarch larvae. The SAP recommended testing *Tetraopes* (red milkweed) beetles as a more logical choice than the monarch butterfly. **Classification: Supplemental** **(Reviewed in U.S. EPA (2002a, 2002e, 2003d, and 2003g))** *Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.	455382-05
N/A	Insecticidal Activity Spectrum Study	Bioassays of six Families of the Order Coleoptera and two Lepidoptera species detected activity only against beetle species of the family Chrysomelidae (corn rootworm and Colorado potato beetle). **Classification: Supplemental** **(Reviewed in U.S. EPA (2003g))**	455328-07
N/A	Field Evaluation of Cry3Bb1 Corn Exposure on Non-Target Organisms	Preliminary results from two-year Tier IV field census studies. These studies are supplemental to Tier I maximum hazard dose testing. The data do not show any MON 863 corn-related adverse effect on non-target and beneficial invertebrate abundance in the field. **Classification: Supplemental** **(Reviewed in U.S. EPA (2002a, 2002e, 2003d, and 2003g))** *Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.	455382-06

Guideline Number	Study	Results	MRID Number
N/A	Non-Target Organism Field Scale Risk Assessment	Final report for MRID No. 455382-06 two-year field census study. MON863 showed no overall differences in the abundance of non-target invertebrates and had less impact on certain beneficial insects compared to traditional insecticides, especially soil and foliar applications. These studies are supplemental to Tier I maximum hazard dose testing and are of inadequate statistical power for a long-term effects determination. **Classification: Supplemental** **(Reviewed in U.S. EPA (2003d and 2003g))** *Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.	457916-01
N/A	Field and Laboratory Invertebrate Studies	Summary (without data) of preliminary findings from several one-year, higher tier field and laboratory studies not triggered by Tier I maximum hazard dose testing data. Final report of studies to be submitted. **Classification: Supplemental** **(Reviewed in U.S. EPA (2002b, 2002e, 2003d, and 2003g))** *Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.	456530-03
885.5200	Aerobic Soil Degradation of the Cry3Bb1 Protein 11098	Finely ground corn leaf tissue in sandy loam field soil degradation data at worst-case field concentrations show that the Cry3Bb1 protein half-life (DT_{50}), based on insect bioassays and enzyme-linked immunosorbent assay (ELISA), was 2.37 and 2.76 days, respectively. The time until 90% decay (DT_{90}) estimates for the insect bioassays and ELISA were 7.87 and 9.16 days, respectively. At ≤28 days, the CryBb1 protein was below the detection level. These results verify that the Cry3Bb1 protein degrades rapidly and does not accumulate in the soil. Additional testing in different soil types is requested. **Classification: Supplemental** **(Reviewed in U.S. EPA (2002c, 2002e, and 2003g))** *Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.	451568-04

Guideline Number	Study	Results	MRID Number
885.5200	Aerobic Soil Degradation of Cry3Bb1 Produced by CRW-Protected Corn Event MON 863	The DT_{50} values for Cry3Bb1in several dosing regimes and soil types ranged from 0.6 days to 2.3 days, and the DT_{90} values ranged from 4.03 days to 50 days. Cry3Bb1 levels in soil sample extracts show that concentrations were near or below the ELISA limit of quantitation (LOQ) (0.16 µg/g) after 2 months of incubation. Additional studies with whole plant tissue are requested. **Classification: Supplemental** **(Reviewed in U.S. EPA (2002g and 2003g)** *Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.	457571-02
N/A	Endangered Species Impact Assessment	Monsanto conducted a hazard assessment, exposure assessment, and risk characterization to demonstrate that Cry3Bb1 does not pose a risk to endangered Coleoptera. The Agency performed an independent assessment and has determined that Cry3Bb1 Event MON 863 will not result in a "may effect" for endangered and/or threatened species listed by the U.S. Fish and Wildlife Service, including mammals, birds, terrestrial and aquatic plants, and invertebrate species. Therefore, no consultation with the U.S. Fish and Wildlife Service is required under the Endangered Species Act. **Classification: Acceptable** **(Reviewed in U.S. EPA (2002a, 2002e, and 2003g))**	455770-03

I. Non-Target Wildlife Study Summaries

a. Mammalian Wildlife

Mammalian wildlife exposure to Cry3Bb1 protein is considered likely; however, the mammalian toxicology information gathered to date on *Bt* Cry proteins does not show a hazard to wild or domesticated mammals. The Cry3Bb1 toxicity data, as described in the Human Health Assessment (see section II(B)(2) of this BRAD), indicate that there is no significant toxicity to rodents from acute oral testing at the maximum hazard dose. Therefore, no hazard to mammalian wildlife is anticipated, and data on wild mammal testing is not required.

b. Avian Species

i. Northern Bobwhite Study (Master Record Identification Number (MRID No.) 449043-15)

The dietary LC_{50} value for Cry3Bb1 corn grain (MON 853, MON 854, and MON 855), when fed to juvenile northern bobwhite for 5 days, was reported to be greater than 70,000 ppm (10% of the diet), the only concentration tested. No adverse effects on bobwhite quail were seen in eight days. These data show that there will be no hazard to avian wildlife from incidental field exposure to Cry3Bb1 corn. These data, however, are not sufficient to make a hazard assessment from repeated exposure to higher doses of Cry3Bb1 corn. The concentration tested (10% corn in the diet) is too low. A six-week broiler study with 60%–70% MON 863 corn in the diet is required to assess hazard to non-target birds from continuous exposure to high levels of Cry3Bb1 protein.

*Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.

c. Aquatic Species

There is no evidence for sensitivity of aquatic (including endangered) species to Cry proteins. Toxicity studies with Cry proteins on aquatic organisms show no hazard for fish or invertebrates exposed to either corn pollen or to bacterially expressed Cry protein. In addition, aquatic exposure from *Bt* corn is extremely small. When a simple standard pond scenario (1-hectare pond, 2 meters deep draining a 10-hectare watershed planted with corn) was used to develop a worst case EEC for Cry3Bb1 protein on the basis of corn pollen loadings from airborne pollen deposition and agricultural runoff from corn plant tissue left in the field at the end of harvest (assuming that no degradation of the protein takes place), airborne and agricultural runoff is calculated to be 3.9 nanograms (ng) Cry3Bb1 protein/mL. Thus, total water concentration of less than 3.9 ng Cry3Bb1 protein/mL is projected under worst-case conditions.

i. Freshwater Fish (MRID No. 449043-19)

The Harmonized Testing Guidelines requirement for a static-renewal freshwater fish toxicity study is usually waived based on low to nonexistent exposure to Cry protein produced in corn. Exposure from corn pollen, if it does take place, will be of a very short duration and quantity and is not expected to have any detectable effect on freshwater fish. Nevertheless, a subchronic eight-week farmed channel catfish feeding study was performed and submitted for review. The study was conducted in compliance with EPA FIFRA Good Laboratory Practice Regulations (40 CFR Part 160) with three minor deviations, none of which had an impact on the integrity of the study.

The study is scientifically sound, and no treatment mortality or behavior change was observed among channel catfish fed diets containing finely ground corn grain from two insect-protected Cry3Bb1 corn lines (MON 853 and MON 859) for eight weeks. The results indicate that corn grain derived from the two transgenic lines producing Cry3Bb1 can be used as a feed ingredient in channel catfish diets at

levels of up to 35% without adverse effect on fish growth, feed conversion efficiency, survival, behavior, or body composition. Significant differences were observed only as lower percentage fillet moisture among fish fed corn grain of the line MON 859; however, these are relatively unremarkable and are unlikely related to the different diets. There were no significant differences noted in feed consumption, weight gain, feed conversion ratio, survival, and percentage visceral fat, or percentages fat, protein, or ash in fillets of channel catfish fed the different test diets. No abnormal fish behavior was observed in the study.

In view of the lack of demonstrated toxicity to channel catfish and minimal aquatic exposure, no freshwater fish hazard is expected from the uses of Cry3Bb1 protein in corn crops.

ii. Freshwater Aquatic Invertebrates (MRID No. 449043-18)

This study was conducted according to procedures specified in Series 72 of EPA's Registration Guidelines, Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation for acute toxicity testing of pesticidal substances to freshwater aquatic invertebrates.

The study was performed on *D. magna*, a freshwater invertebrate. The test material consisted of corn pollen from corn plants (line MON 858). The Cry3Bb1 content was estimated to be 18.8 µg/g fresh weight pollen. The study was procedurally sound, and no treatment mortality or behavior change was reported between the dosed and control replicates for the 48-hour exposure period.

The October 2000 and August 2002 SAP reports recommended that non-target testing be focused on species exposed to the crop being registered. The Agency has determined that the non-target organisms most likely to be exposed to the protein in transgenic corn fields are beneficial insects feeding on corn pollen and nectar, and soil invertebrates, particularly coleopteran species. Therefore, testing of aquatic invertebrates was performed primarily to satisfy the testing requirements for microbial toxins published in 40 CFR Part 158. No substantial aquatic exposure to Cry3Bb1 protein contained within corn plant tissue is expected, except for possibly small amounts of pollen. Several public comments have raised questions about using corn pollen in aquatic invertebrate testing with *D. magna* because corn pollen is thought to be too large for ingestion by these filter feeders. However, there is some observational evidence that daphnids do ingest pollen. As indicated in some study reports reviewed by the Agency, daphnids were actually yellow in color, which can be indicative of ingestion of the yellow pollen test material. However, there is no clear evidence that *D. magna* is capable of ingesting particles as large as pollen. Therefore, only a statement of no effect from exposure to pollen, and no statement on lack of toxicity can be made from this study. Nonetheless, since the Cry3Bb1 protein is confined to corn tissue, and the worst case aquatic EEC is calculated to be 3.9 ng Cry3Bb1 protein/mL, there is no substantial exposure to aquatic invertebrates; no hazard from the registered used of Cry3Bb1-containing corn is anticipated. As a result, no further aquatic invertebrate testing is required at this time.

*Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.

iii. Estuarine and Marine Animals

The estuarine and marine animal studies are waived for this product because of very low to no potential for exposure to CryBb1 protein from field corn.

d. Terrestrial and Aquatic Plants

Since the active ingredient in this product is an insect toxin (*Bt* endotoxin) that has never shown any toxicity to plants, these studies have been waived for this product. Outcrossing issues are addressed in section II(C)(2)(a)(ii)(V) of this BRAD.

e. Non-Target Insect Testing

The October 2000 SAP concluded that invertebrates, such as earthworms and springtails (collembola), are appropriate indicator species for Cry protein testing because of the specific nature of the Cry protein toxicity to select target species. When it initially reviewed the applications for PIP products that were registered in 1995, EPA considered requiring studies evaluating effects upon the representative beneficial soil invertebrates, collembola and earthworms. The Agency was concerned (1) that such soil organisms may be subject to long-term exposure as a result of soil incorporation of crop residues or when crop residues are left on the soil surface and (2) that adverse effects on such soil organisms could result in an accumulation of plant detritus in fields. Recent reports of exudation of Cry proteins by corn roots throughout the growing season add to this concern. The Agency understands, however, that routine agronomic practices have included the long-term use of chemical insecticides, which have adverse effects on soil organisms, but there has not been an accumulation of significant amounts of plant detritus in soils. Thus, Cry3Bb1 corn, which is expected to have less impact on these species than chemical pesticides, should not result in any increased build up of plant detritus or Cry proteins at toxic levels. Supporting this conclusion are data received by EPA that indicate that such proteins are known to degrade rapidly in field soils. Cry proteins that are bound to soil particles have been shown to be rapidly degraded by soil microbes upon elution from the soil particles. Therefore, the potential for significant soil buildup and adverse effects to non-target soil organisms are not anticipated. It has been confirmed in published literature that *Bt* Cry protein released from root exudates and biomass of *Bt* corn plants has no apparent effect on earthworms, nematodes, protozoa, algae, bacteria, actinomyces, and fungi in soil in spite of the fact that enough detectable Cry protein is bound to soil particles to show toxicity to the target pest. These results suggest that, despite its presence in soil, the Cry protein released in root exudates of *Bt* corn, or from the degradation of the biomass of *Bt* corn, is not toxic to a variety of organisms in the soil environment. It has also been reported that the same degree of *Bt* Cry protein persistence takes place in soils that have been exposed to repeated *Bt* microbial spray applications. In addition, new plants grown in *Bt*-containing soil do not take up the *Bt* protein. Nevertheless, data on insects closely related to the target pest, as well as other studies to address the published data requirements for registration of microbial toxins (40 CFR Part 158), have been received and reviewed.

i. Effects on Honey Bee Larvae (MRID No. 449043-10)

An acceptable study was conducted based on OCSPP Harmonized Test Guideline 885.4380 (Honey Bee Testing, Tier I). This study was conducted in accordance with Good Laboratory Practice Standards (40 CFR Part 160) with certain exceptions that did not affect the integrity of the test.

Testing was conducted with *Bt* Cry 3B2.11231 protein (purity 96%; 1.79 mg active protein/mL water; current nomenclature refers to this protein as Cry3Bb1) inoculated directly into larval brood cells prior to capping. Within 18 days after treatments were administered, all larvae emerged from capped brood cells. All larvae (100%) treated with Cry3Bb1 protein survived to pupation or "capping"; whereas, 97.5% (2.5% mortality) of the honey bee larvae in the control group survived to pupation. There was no statistical difference ($p = 0.05$) in total percent mortality during the larval development or adult emergence stages between treated and control groups. Based on the results presented in the study, it can be concluded that honey bee development and survival are not affected by exposure to the Cry3Bb1 protein. There was 88.8% mortality of larvae treated with the reference substance, potassium arsenate, which indicated that bees were exposed to the treatments. The LC_{50} for honey bee larvae was determined to be >1,790 ppm Cry3Bb1 protein.

According to the OCSPP Harmonized Testing Guidelines, non-target insects should be tested at 10–100x the field dosage. This test was conducted at an acceptable level 100x the concentration in pollen or 1,790 ppm Cry3Bb1 protein. Since potential exposure of honey bees to Cry3Bb1 will be from pollen, this test was conducted at an appropriate maximum hazard dose. Therefore, no hazard to honey bee larvae and their development is expected from exposure to the Cry3Bb1 protein in corn pollen.

ii. Adult Honey Bee Testing (MRID No. 449043-11)

An acceptable study was conducted based on OCSPP Harmonized Test Guideline 885.4380 (Honey Bee Testing, Tier I). This study was conducted in accordance with Good Laboratory Practice Standards (40 CFR Part 160) with certain exceptions that did not affect the integrity of the test.

The testing consisted of a control group fed 30% sucrose in deionized water, a reference group fed 100µg/mL potassium arsenate, a test group fed 360 µg/mL of Cry3Bb1 protein, and a water only group. The study concluded that 360 µg/mL Cry3Bb1 protein did not affect survival or behavior of adult honey bees. The maximum hazard dose, an LC_{50} greater than 360 µg/mL, is 20x the concentration found in pollen. Therefore, no hazard to adult honey bees is expected from exposure to the Cry3Bb1 protein in corn pollen.

iii. Parasitic Hymenoptera Testing (MRID No. 449043-13)

This study was conducted based on OCSPP Harmonized Test Guideline 885.4340 (Non-Target Insect Testing, Tier I). This study was conducted in accordance with Good Laboratory Practice Standards (40 CFR Part 160) with certain exceptions that did not affect the integrity of the test.

A dietary toxicity study with the parasitic Hymenoptera (*N. vitripennis*) was conducted with *Bt* Cry 3B2.11231 (Cry3Bb1) protein (purity 96%; 34.5 mg active protein/mL water). Wasps were tested at rates of 400 and 8,000 ppm Cry3Bb1 protein, which is approximately equivalent to 1x and 20x the maximum protein concentration in plant tissue. The LC_{50} for parasitic Hymenoptera was determined to be >8,000 ppm Cry3Bb1 protein. When an adjustment for mortality in the control group is considered, mortality in the 8,000 ppm treatment group is 45%. Although differences in mortality between the control and treatment groups were not significantly different ($p>0.05$), a treatment effect at 20x EEC could not be precluded in this study. At test termination, mortality for the 100 ppm potassium arsenate reference group was 33% (24 of 73) and mortality for the 1,000 potassium arsenate reference group was 100% (70 of 70).

Based on this test, the LC_{50} for adult parasitic Hymenoptera exposed to dietary Cry3Bb1 is >8,000 ppm. The hazard assessment is based on 4,000 ppm Cry3Bb1 protein, which is 10x the field concentration in plants. However, because parasitic Hymenoptera do not feed directly on corn plant tissues, minimal exposure of parasitic Hymenoptera to Cry3Bb1 protein is expected. As a result, no hazard to *N. vitripennis* is expected from exposure to MON 863 Cry3Bb1 corn.

The preliminary review of the *N. vitripennis* study was initially found acceptable by the Agency (U.S. EPA 2002a). However, the August 27, 2002 SAP concluded that the parasitic Hymenoptera (*N. vitripennis*) testing was not appropriate. The SAP concluded that "[t]he levels of exposure of...*Nasonia* to active protein were not, for example, determined throughout their respective tests. The test protein...within a diet broth...could have degraded considerably." Not only were the procedures in this study questioned by the SAP, the appropriateness of testing this organism was questionable. *N. vitripennis* is a dipteran parasitoid that does not occur in corn fields. A more appropriate parasitoid that occurs in corn fields (e.g., *Tricogramma* or *Macrocentrus grandii*) should be considered. Since *Tricogramma* and *Macrocentrus* are lepidopteran parasitoids, testing another beneficial organism rather than a parasitoid is appropriate. Therefore, the Agency is requiring additional maximum hazard dose laboratory testing of a beneficial coleopteran, such as a carabid (ground beetle).

*Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.

iv. Green Lacewing Larva Testing (MRID No. 449043-12)

This study was conducted based on OCSPP Harmonized Test Guideline 885.4340 (Non-Target Insect Testing, Tier I). This study was conducted in accordance with Good Laboratory Practice Standards (40 CFR Part 160) with certain exceptions that did not affect the integrity of the test.

Green lacewing larvae were fed the Cry3Bb1 protein in a moth egg (*Sitotroga* spp.) and water meal diet at rates of 400 and 8,000 ppm, which is approximately equivalent to 1x and 20x the maximum protein concentration in plant tissue. There was 20% mortality in the negative control group on day 10. Compared to the negative control, at day 10, there was no significant increase in green lacewing larval

mortality when fed 1x (400 ppm) and 20x (8,000 ppm) the maximum Cry3Bb1 protein concentration found in plant tissue. At test termination, mortality was 43% (13 of 30) for the 1,000 ppm reference group and 100% for the 10,000 ppm reference group (potassium arsenate). The data show that the LC_{50} for green lacewing larvae exposed to Cry3Bb1 in diet is >8,000 ppm. Based on these results, it is not expected that the green lacewing will be adversely affected when exposed to Cry3Bb1 in the field.

The preliminary review of the green lacewing larva study was initially found acceptable by the Agency. However the August 27, 2002 SAP concluded that the green lacewing (*Chrysoperla carnea*) testing was not appropriate. Several public comments also addressed this issue. The SAP concluded that "[t]he levels of exposure of *Chrysoperla* to active protein were not, for example, determined throughout their respective tests. The test protein was held for a week within a diet broth in the *Chrysoperla* test chamber, and could have degraded considerably."

Additional problems were recognized with the *Chrysoperla* laboratory study. Green lacewing are difficult to test in the laboratory because of a high rate of mortality. In this instance (MRID No. 449043-12), the test was terminated after 10 days because there was >20% mortality in the negative control. In addition, it is questionable whether the green lacewings were ingesting the Cry3Bb1 protein that was coated around moth eggs in a diet. Since green lacewing have piercing-sucking mouthparts, they may not be exposed to the protein on the external surface of the egg diet. Therefore, Monsanto must conduct a laboratory insect toxicity test on an alternate organism. The minute pirate bug (*Orius insidiosus*) would be a more appropriate species to test than the green lacewing. *Orius* typically occur in corn fields as egg predators and they typically feed on pollen. Therefore, a laboratory study, feeding *O. insidiosus* both pollen and purified protein in diet, is required. Feeding *O. insidiosus* Cry3Bb1 protein in diet will allow for a test at the maximum hazard dose; whereas, feeding *O. insidiosus* pollen expressing the Cry3Bb1 protein will provide an evaluation of potential effects from actual exposure scenarios.

*Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.

v. Lady Beetle Testing

Since the Cry3Bb1 protein specifically targets coleopteran (beetle) insects, particular attention is warranted regarding potential effects of MON 863 on lady beetles. In addition to a dietary exposure study to the purified Cry protein, the Agency requested a test demonstrating the effect on lady beetles feeding on corn pollen containing Cry3Bb1. Monsanto conducted three additional laboratory studies on two different lady beetle species (*C. maculata* and *H. convergens*) in response to this request.

Adult Lady Beetle Protein Dietary Study (MRID No. 449043-14)

A diet containing purified Cry protein and honey was fed to the adult lady beetle (*H. convergens)* at rates one and 20 times the maximum protein concentration found in corn leaf tissue. When the negative control group reached 20% mortality on day 10, the results showed no significant differences in the

mortality rate between lady beetles fed 400 and 8,000 µg Cry3Bb1/mL of diet. Results from this study showed that the LC_{50} for Cry3Bb1, when incorporated in diet and fed to *H. convergens*, is >8,000 µg Cry3Bb1 protein/mL diet. Mortality for the 1,000 and 10,000 µg potassium arsenate/mL diet groups were 55% and 95%, respectively, at day 10. This demonstrates that toxicity can be measured by mixing a test substance in the lady beetle diet. Lady beetles do not feed on corn plant tissue. They do, however, feed on corn pollen and prey on pest insects that may feed on corn tissue and contain Cry3Bb1 in their gut, thus resulting in exposure to the *Bt* protein. There is approximately 390 µg Cry3Bb1/gram (g) fresh weight corn tissue. Lady beetle exposure is expected to be significantly lower than this since the corn tissue would be metabolized, eliminated, or otherwise degraded within the prey species. Since the maximum hazard dose LC_{50} was found to be 8,000 µg Cry3Bb1/mL diet, which is 20 times higher than maximum expected exposure levels, no hazard from Cry3Bb1 in corn plants to adult lady beetles is anticipated.

Larval Lady Beetle Pollen Feeding Study (MRID No. 455382-04)

At certain times, corn pollen may comprise up to 50% of lady beetle larva's diet. Therefore, the effects of corn pollen containing event MON 863 Cry3Bb1 protein on lady beetle larvae (*C. maculata*) was evaluated. Pollen was fed to lady beetle larvae in a diet consisting of equal amounts of lyophilized tephritid fruit fly eggs and bee pollen. Diets contained 50% pollen (93 µg Cry3Bb1/g fresh pollen weight), since this is the potential level of field exposure, and an equal amount of the tephritid fruit fly diet. First instar lady beetle larvae were individually placed in test arenas to avoid cannibalism. There was not a statistically significant difference between developmental time of larvae to pupae and/or adults, nor was there a difference in adult weight survival between larvae fed bee pollen or corn pollen. There was also no difference between larvae fed *Bt* and non-*Bt* pollen. There was a significant difference between the reference group (potassium arsenate) and other test groups since no larvae survived in the reference group. The 100% mortality observed in the reference group verified that the lady beetles were ingesting the diet. This test was conducted with pollen levels greater than or equal to levels lady beetle larvae are expected to be exposed to in the field. Therefore, the LC_{50} for Cry3Bb1 expressed in corn pollen is >93 µg/g fresh pollen weight. This study demonstrates that lady beetle larvae will not be adversely affected by Cry3Bb1 field corn.

***Adult Lady Beetle Pollen Feeding Studies* (MRID Nos. 453613-01 and 453613-02)**

C. maculata lady beetle adults were fed diets of transgenic corn pollen mixed with fruit fly eggs to determine the potential effects of transgenic pollen to beetles (MRID No. 453613-01). The corn (MON 863) test pollen (assayed at the time of testing) contained the Cry3Bb1 protein at a concentration of 37.4 µg/g pollen. After 30 days of diet exposure, 83.3% and 80.0% of adult *C. maculata* survived in the test and control pollen groups, respectively. While these survival rates were significantly less than that in the assay control group (bee pollen, which exhibited 100% survival), there were no significant differences between the test and control pollen groups. All adults in the positive control (arsenate-treated corn pollen) died in less than 8 days. Results indicated that transgenic *Bt* corn pollen expressing the variant Cry3Bb1 protein have no significant negative effects on the survival of *C. maculata* adults.

H. convergens adults were fed diets of transgenic corn pollen in honey to determine the potential effects of transgenic pollen to non-target beetles (MRID No. 453613-02). The corn (MON 863) test pollen (assayed at the time of testing) contained the Cry3Bb1 protein at a concentration of 37.4 µg/g pollen. After 15 days of diet exposure, 84% and 81% of adult *H. convergens* survived in the test pollen and control pollen groups, respectively. There were no significant differences in survival among the test pollen, control pollen, and the assay control (honey only) treatment groups. Only 5% of beetles exposed to the positive control (arsenate-treated corn pollen) survived. Results demonstrate that transgenic *Bt* corn pollen expressing the variant Cry3Bb1 protein had no significant negative effects on the survival of *H. convergens* adults from dietary exposure.

No adverse effects were detected when *C. maculata* and *H. convergens* were fed MON 863 pollen in diet in the laboratory. Pollen levels consumed by the lady beetles in this study exceeded concentrations that are expected to be encountered in the field. Therefore, it can be concluded MON 863 will not pose a hazard to lady beetle adults in the field.

vi. Collembola Feeding Study (MRID No. 449043-17)

This study was conducted based on OCSPP Harmonized Test Guideline 885.4340 (Non-Target Insect Testing, Tier I). This study was conducted in accordance with Good Laboratory Practice Standards (40 CFR Part 160) with certain exceptions that did not affect the integrity of the test.

Collembola (*Folsomia candida*) were fed diets consisting of transgenic corn leaf tissue containing Cry3Bb1 protein mixed with dry granulated Brewer's yeast. Diets contained a ratio of 0.50, 5.0 and 50% corn leaf tissue in Brewer's yeast, which was equivalent to 8.73, 87.3 and 872.5 µg Cry protein/g diet, respectively. The corn leaf tissue contained 1,745 µg Cry3Bb1 protein/g dried leaf tissue.

These results show a LC_{50} >872.5 µg/g diet of Cry3Bb1 protein. The study also noted that a diet containing 50% corn leaf tissue expressing the Cry3Bb1 *Bt* protein (a maximum hazard dose) did not adversely affect reproduction of collembola. This test was conducted at concentration levels much greater than collembola are expected to be exposed to in the field. The primary route by which collembola would be exposed to Cry3Bb1 in the field is through decaying root tissue (and possibly from pollen to a much lesser degree). MON 863 is expressed in corn roots in the range of 3–66 µg/g, which is significantly lower than the levels used in this test.

This study adequately addresses potential concerns for Cry3Bb1 protein expressed in transgenic corn to collembola (*F. candida*), a representative of beneficial soil insect species. The results of this study demonstrate that Cry3Bb1 protein found in transgenic corn poses no hazard to soil-inhabiting collembola species and, by inference, to other beneficial non-coleopteran soil insects. Notably, one of the October 2000 SAP's recommendations was that invertebrates of different orders than those known to be affected by the Cry protein in question not be tested.

vii. Earthworm Toxicity Testing

Earthworm feeding studies submitted to the Agency for all of the registered Cry proteins demonstrate that the Cry proteins are not toxic to earthworms at the worst-case environmental concentration. Some public comments have voiced concerns as to whether the earthworms actually ingested the *Bt* Cry proteins when these are incorporated into the soil in the test systems used. Recently published data show that the earthworms do, however, ingest the *Bt* Cry proteins with the soil without harmful effects. The data also show that there were no significant differences in the percent mortality and weight of earthworms after 40 days in soil planted with *Bt* or non-*Bt* corn, in fallow fields, or after 45 days in soil amended with biomass of *Bt* or non-*Bt* corn or not amended. The *Bt* Cry protein was shown to be present in both the casts and guts of the worms.

- **MRID No. 449043-16** – This study complied with Good Laboratory Practice Standards (40 CFR Part 160) and OECD Principles of Good Laboratory Practice with certain exceptions that did not affect the integrity of the test. The testing was conducted based on OCSPP Harmonized Test Guideline 850.6200 (Earthworm Subchronic Toxicity Test) and OECD Guideline 207. This study meets current testing requirements for assessing risks to earthworms from plant-incorporated protectants derived from *Bt*.

  The 14-day LC_{50} for earthworms exposed to Cry3Bb1 protein 11231 in an artificial soil substrate was determined to be greater than 570 mg Cry3Bb1 protein/kg dry soil. However, the percent mortality reported was 38%. The mortality in the 57.0 milligrams per kilogram (mg/kg) group was 8%. It was noted in the study design that the levels of buffer salt in the test groups were higher than expected because of a miscalculation. The actual concentration of sodium bicarbonate salt in the 57.0 and 570 mg Cry3Bb1 protein/kg treatment groups was 70 and 699 mg/kg, respectively. The higher concentrations did not appear to have any influence on the overall conclusions of the study. Nonetheless, another earthworm study (MRID No. 457571-01) was performed.

- **MRID No. 457571-01 –** The submitted study is classified as acceptable, is scientifically sound, and is consistent with current testing requirements for earthworm hazard assessment. The 14-day LC_{50} for earthworms exposed to purified 11098 Cry3Bb1 (*Escherichia coli*-produced) protein in an artificial soil substrate was determined to be greater than 166.6 mg/kg dry soil (the highest concentration tested), or greater than 20 times the worst-case EEC in a corn field. There was no apparent effect of the phosphate buffer on the earthworms. There were no earthworm mortalities in any of the controls or Cry protein-treated soils during the 14-day study. Changes in average body weights were not statistically different ($p>0.05$) among the controls and protein-amended soils. There were no other remarkable observations. At the end of the study, mortality in the 10 and 20 mg chloroacetamide/kg soil was 2.5% (1 of 40) and 85% (34 of 40), respectively. Percent mortality of earthworms in the reference substance (chloroacetamide) groups was consistent with historical results and further confirmed the adequacy and consistency of the protocol used in the definitive test.

The reviewed data show that no adverse effects to earthworms are expected in fields growing Cry3Bb1 corn plants.

viii. Monarch Butterfly Larval Pollen Feeding Study (MRID No. 455382-05)

This study was not required nor requested for Cry3Bb1 because it is a coleopteran-active protein that is not expected to affect lepidopterans such as the monarch butterfly. In addition, extensive research conducted on the potential affects of monarch feeding on lepidopteran-active *Bt* corn pollen has shown a lack of concern for subchronic toxicity. Due to recent public concern for possible adverse effects of Cry3Bb1 corn on monarchs, Monsanto sponsored this study and submitted it to the Agency for review. This study has demonstrated that corn pollen expressing the Cry3Bb1 protein will not result in subchronic toxic or developmental effects to monarch larvae. Neonate monarch survival was not affected after feeding on milkweed dusted with up to 3,200 pollen grains/square centimeter (cm^2) expressing Cry3Bb1 for 2, 4, or 10 days of pollen exposure. Larval development, weight gain, and milkweed leaf consumption were also not affected by feeding on *Bt* pollen 96 hours and 10 days after exposure. Pollen densities in the field are not expected to be as great as 3,200 grains/cm^2. Pollen densities in the field average 150 grains/cm^2. Levels of 400 and 800 pollen grains/cm^2 would probably be rare. Therefore, results of this study indicate that young monarch larvae (at the most sensitive stage) will not be adversely affected by exposure to corn pollen expressing Cry3Bb1 in the field. Since Cry3Bb1 expressed in MON 863 is a coleopteran-active protein, however, the August 27, 2002 SAP concluded that the monarch butterfly was not an appropriate indicator organism to be tested. The SAP recommended testing *Tetraopes* (red milkweed beetles) as a more logical choice than the monarch butterfly.

*Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.

ix. Insecticidal Activity Spectrum Study (MRID No. 455328-07)

Insecticidal spectrum of activity bioassays were conducted on six Coleoptera Families and two Lepidoptera species. A series of six to eight concentrations of CryBb1 protein (1 ppm–8,000 ppm) in standard insect diet preparations were used to conduct seven-day mortality testing. Significant insecticidal activity was seen only in the family Chrysomelidae (corn rootworm and Colorado potato beetle) of the Order Coleoptera. No activity was seen against the cowpea weevil (Bruchidae), lady bird beetle (Coccinellidae), red flour beetle (Tenebrionidae), cotton boll weevil, pepper weevil, and rice weevil (Curculionidae). The Lepidoptera corn earworm (Noctuidae) and European corn borer (Crambidae) were also not affected. This efficacy study was reviewed for environmental assessment purposes to expand the number of insect species examined for possible toxicity of the Cry3Bb1 protein. The results confirm the assertion that *Bt* Cry proteins have a very specific and narrow range of target species.

x. Field Evaluation of Cry3Bb1 Corn Exposure on Non-Target Invertebrates

The Scientific Advisory Panels (October 2000 and August 2002) recommended that non-target testing be focused on species exposed to the crop being registered. The Agency has determined that the non-target organisms most likely to be exposed to the Cry3Bb1 protein in transgenic corn fields were beneficial insects feeding on corn pollen and nectar, and soil invertebrates, particularly coleopteran species. In addition to extensive and difficult maximum hazard dose single species soil coleopteran toxicity testing followed by an extrapolation from the results to a community risk assessment, and the fact that all of the species cannot be tested in the laboratory, direct field test and field census data on coleopteran insect effects and abundance were requested, received, and evaluated. These studies were conducted in several states by Monsanto and several independent university scientists. Results of these studies are summarized below. Some of the submissions consist of preliminary results of studies in progress. The Agency requested that Monsanto submit the final reports of these studies as they become available.

These preliminary field test and field census data with the study design methodologies have been presented to an SAP (August 2002). The SAP commented that the study designs lack appropriate statistical power, but that methodology for conducting statistically valid field census studies at the scale necessary to determine ecosystem effects is not available. Such methodology is yet to be developed. As a result, the Agency is reviewing the available field studies as data supplemental to the maximum hazard dose single species laboratory testing but useful for short-range assessment of non-target invertebrate abundance in Cry3Bb1 corn test plots. It is an accepted practice in the Office of Pesticide Programs to use the trends seen in several supplemental studies for hazard assessment when a perfect study is not available.

a) Preliminary Invertebrate Field Census Data (MRID No. 455382-06)

These two-year Tier IV field studies are intended to supplement the Tier I maximum hazard dose findings. Invertebrates in Cry3Bb1 corn field plots were sampled from the soil, soil surface, and foliage. Soil-dwelling invertebrates were collected using a "pan trap," which utilized a modified Burlese extraction method. Surface-dwelling invertebrates were sampled in the field with pitfall traps. Foliage-dwelling invertebrates were monitored by yellow sticky traps (Pherocon AM™) set in the field at canopy level and adjusted as the season progressed. Sampling for lady beetles was also done using a drop-cloth technique. Preliminary results do not show any MON 863 corn-related adverse effect on non-target and beneficial invertebrate abundance in the field. The final report (MRID No. 457916-01) is discussed below.

b) Final Invertebrate Field Census Data (MRID No. 457916-01)

Methods:
During the 2000 and 2001 growing seasons, event MON 863 CRW-protected corn and non-transgenic corn (hybrid RX670) were grown in Warren County near Monmouth, Illinois. Corn was planted in both fields the previous year and soybeans were planted two years prior to conducting these field trials. All

experimental plots were managed according to typical cultural practices of commercially grown corn in the region and included the application of the herbicides acetochlor and atrazine after planting and before emergence.

Bt (MON 863) and control hybrids (RX670) were the main plots planted in a split-plot design with four replications planted 20 feet (ft) apart. Rows were planted 30 inches apart, seeded at a rate of approximately 1.7 seeds/ft, and planted 1.5–1.75 inches deep. Plots (240 ft × 60 ft) were divided into 24 row subplots (60 ft × 60 ft) that served as replications receiving one of 4 insecticide regimes. Insecticide treatments of the *Bt* and non-*Bt* plots included the following:

(1) No insecticide
(2) Seed treated with Gaucho® prior to planting
(3) Granular insecticide, Force 3G®, applied and incorporated in furrows at planting
(4) Foliar insecticide, Pounce 3.2 EC®, applied at the V10 and R2 corn growth stages to control 1st and 2nd generation CRW adults.

A four-row buffer of non-transgenic corn was planted around each plot to minimize edge effects from adjacent subplots.

Data were collected on agronomic and phenotypic characteristics, pest (insect and disease) susceptibility, soil quality and fertility, microbial populations, and non-target invertebrate abundance. Eight-inch deep soil samples were taken to evaluate quality and fertility. Four samples were taken during the growing season and two were taken post-harvest in 2000; two samples were taken during and after the growing season in 2001 for a total of ten samples. Microbial populations were evaluated from test and control plots that received no insecticide regime a total of 14 times during the growing season from the top six inches of soil within six inches of the rhizosphere. Samples were taken in 2000 and 2001 during the V2, V4, V8, R1, and R6 stages, as well as after tillage and the following spring. Soil samples from the 2000 growing season were analyzed for bacteria, mold, and yeast by a heterotrophic plate count method. Four of the seven soil samples collected during the 2001 growing season were analyzed for bacteria, actinomycetes, and fungi by the "viable plate count method."

Soil-dwelling invertebrates were collected from root balls, including soil during the V6, V10, and R1 growth stages during the 2000 and 2001 growing seasons. Three eight-inch root balls were collected during the V6, V10, and R1 corn growth stages from all control and test plots and processed through a Berlese funnel to extract invertebrates. Earthworms were also collected from soil samples by hand sorting. Ground surface-dwelling invertebrates were collected from all test and control plots under all insecticide regimes using pitfall traps. Four pitfall traps were placed in each subplot from the V6 to R4 corn growth stages for three-day periods. Key invertebrates from pitfall traps were counted and identified to family level. Flying and foliage-dwelling invertebrate were collected from each subplot using yellow sticky traps. Three traps per subplot were placed at canopy level from the V6 to R4 corn growth stages. Traps were left in the field for seven days and all key taxa were counted and identified to

family or genus level. Data were analyzed using a mixed linear repeated measures model for each invertebrate collected by each sample method.

Results:
Among the three sample methods (soil, pitfall, and sticky trap), there was a total of 156,572 organisms from 16 orders and 36 families identified during the 2000 and 2001 growing seasons. Collected invertebrates included pests, predators, parasitoids, detritivores, and decomposers. The predominant non-target invertebrates collected in each sample method are summarized in Table 2.

Table 2. Predominant Non-Target Invertebrates Collected in Each Sample Method.

Sample Method	Order (Family)
Soil and Root Samples (soil-dwelling invertebrate)	Diplura (Japygidae), Chilopoda, Aranea, Acari, Oligocaeta (earthworms), Coleoptera (Carabidae, Staphylinidae, Nitidulidae, Lanthridiidae), Hymenoptera (Formicidae)
Pitfall Trap Samples (ground surface-dwelling invertebrate)	Orthoptera (Gryllidae), Coleoptera (Carabidae, Staphylinidae, Nitidulidae, Scarabeidae, Chrysomelidae), Hymenoptera (Formicidae), Araneae, Chilopoda
Yellow Sticky Trap Samples (flying & foliage-dwelling invertebrate)	Coleoptera (Chrysomelidae, Nitidulidae, Coccinellidae), Hymenoptera, Homoptera (Aphididae, Cicadellidae), Hemiptera (Anthocoridae), Diptera (Syrphidae), Neuroptera (Chrysopidae, Hemerobiidae), Aranea

Overall, there was no statistical difference between MON 863 and non-*Bt* plots (RX670) in the abundance of the predominant invertebrates collected in soil samples. According to the soil sample data, the number of Japygidae (diplurans) did not differ between *Bt* and non-*Bt* plots; however, there were significantly less collected in the insecticide-treated plots. Of the coleopteran insects collected, there were generally more carabids (ground beetles) and staphylinids (rove beetles) than nitidulids (sap beetles) and lanthridiids (minute brown scavenger beetles). There was no statistical difference in the number of coleopteran insects captured between the MON 863 and non-transgenic corn isolines. Statistical analysis showed that insecticide treatments significantly reduced the number of carabids in 2000 and the number of staphylinids on the last sample date in 2001. Invertebrates from the Araneae (spider) and Acari (mite) families were also not significantly different between *Bt* and non-*Bt* plots; however, the number of acarids (mites) was statistically greater in the plots treated with foliar insecticides in 2001. The number of chilopods (centipedes), the most abundant non-insect arthropod sampled, was not different between *Bt* and non-*Bt* isolines but more were collected in plots treated with seed, soil, and foliar insecticides in 2001, particularly the MON 863-treated plots. Although the number of earthworms collected did not differ between *Bt* and non-*Bt* plots, there were significantly less earthworms in the plots treated with foliar insecticides than the other insecticide regimes. The hand-sorting method also showed no differences in the number of earthworms collected in MON 863 and non-transgenic plots, nor was there a difference found between insecticide regimes.

Overall, there was no statistical difference between MON 863 and non-*Bt* plots (RX670) in the abundance of the predominant invertebrates collected in pitfall trap samples. Of the coleopterans

captured in pitfall traps, there were more nitidulids (sap beetles), carabids (ground beetles), and staphylinids (rove beetles) captured than chrysomelids and scarabids collected. There was no statistical difference between MON 863 and RX670 field plots in the overall number of coleopterans collected in pitfall traps. The different insecticide regimes tested resulted in varied and inconsistent effects on the abundance of the predominant Coleoptera sampled. The number of gryllids (crickets) and formicids (ants) collected in pitfall traps was not statically different between *Bt* and non-*Bt* plots or different insecticide regimes. Chilopod (centipede) and Araneae (spider) abundance was not different between MON 863 and RX670 plots nor was there an effect from insecticide regimes in most cases. Statistical analysis showed that there were significantly fewer Araneae (spider) in the plots with soil and foliar insecticide treatments in 2000.

Based on yellow sticky trap counts, there were consistently less corn rootworm (CRW; *Diabrotica virgifera* and *Diabrotica barberi*) in the MON 863 plots than the non-*Bt* plots in all insecticide regimes. The difference in the number of CRW captured, however, was not statistically different. In both 2000 and 2001, there were no significant differences between MON 863 and RX670 plots across all insecticide regimes in the number of nitidulids (sap beetles), coccinellids (lady beetles), aphidids (grass hoppers), cicadellids (potato leaf hoppers), braconids (*Macrocentrus grandii* - a parasitoid), syrphids (syrphid or hover flies), hemerobiids (brown lacewing), chrysopids (green lacewing) and Araneae (spiders). There was a reduction in the abundance of *C. maculata* (lady beetles) and *M. grandii* and an increase in *Empoasca fabae* (potato leaf hopper) in plots receiving a foliar spray. Syrphid fly abundance was reduced in 2000 by soil insecticide treatments.

Field Census Summary:
Data collected during the 2000 and 2001 growing seasons indicate that MON 863 and RX670 corn are agronomically and phenotypically equivalent, and there are not differences in their susceptibility to pathogens. Soil quality and fertility were also found to be consistent among MON 863 and RX670 field plots. Sampling data collected in 2000 and 2001 also showed that there are no overall differences in the abundance of non-target invertebrate collected in MON 863 and RX670 plots. However, corn pests, such as *D. virgifera*, *Chaetochnema pulicaria*, and *Rhopalosiphum maidis*, as well as predators (e.g., *O. insidiosus* and *C. maculata* (lady beetle)), parasitoids (e.g., *M. grandii*), and decomposers (e.g., earthworms and diplurans) were significantly impacted by insecticide regimes. Foliar sprays and soil treatments resulted in the greatest impact on non-target organisms such as carabids, spiders, *O. insidiosus* (a generalist predator), *C. maculata* (lady beetle), and *M. grandii* (parasitic wasp). Therefore, the report concluded: "MON 863 had less impact on certain beneficial insects compared to traditional insecticide control programs, especially soil and foliar applications. Thus, the use of MON 863 for corn rootworm control can lead to reduced use of insecticides and increased compatibility with Integrated Pest Management programs in corn."

Field Census Conclusions:
According to the data submitted to the Agency by Monsanto, MON 863 corn does not adversely impact the abundance of non-target invertebrate found in corn fields. Nonetheless, plot size (240 ft × 60 ft plots divided into 24 row 60 ft × 60 ft subplots) was small and only replicated four times. In addition, each

plot only included three root and sticky trap samples and four pitfall trap samples. The August 2002 SAP concluded that field experiments must be appropriately designed to provide a measure of ecological impacts. In addition, the SAP opinion was that a two-year field study would not be sufficient to determine if MON 863 corn will have long-term impact on non-target invertebrates. Several public comments also expressed this concern. Short-term field studies are not adequate to draw conclusions on the variations in non-target invertebrate populations. Large field-scale studies, conducted for at least three to four years, would be needed to draw a conclusion on non-target impacts. The Panel generally concluded that "the state-of-the-science" needed for long-term studies must improve for the research to be appropriately conducted to provide meaningful results. The statistical power (avoiding Type II experimental error) needed to gain useful results from field studies would require very large fields, more replications, and more samples per plot (e.g., 10 soil and pitfall samples) plus the addition of visual plant samples (e.g., >50/plot). Since the endpoint for field census studies has not been determined, it is difficult to determine how large the fields should be, how many replications are needed, and how many samples per plot are needed to achieve appropriate statistical power. Therefore, additional field census studies should not be conducted until the endpoints and logistics of the study have been determined. If Tier I maximum hazard dose single species laboratory studies show a hazard, intermediate field or semi-field studies between laboratory and full-scale field studies should be conducted. Additional full-scale field or semi-field studies with appropriate end points and statistical power should also be considered based on recommendations of the August 27, 2002 SAP.

The submitted field census data, demonstrating an abundant presence and diversity of invertebrates in the corn CryBb1 corn field, are useful for short-term hazard assessment as supplementary information, which shows the same no-hazard trend seen in the maximum hazard dose single species laboratory testing.

c) Year 2001: Field and Laboratory Invertebrate Studies (MRID No. 456530-03)

A summary of preliminary findings from several one-year supplemental higher tier field and laboratory studies was submitted (MRID No. 456530-03). These studies were not triggered by Tier I maximum hazard dose testing data; however, they appear to have value for assessing possible long-term effects on invertebrate populations. These studies are being conducted in Kansas, Nebraska, Illinois, Virginia, South Dakota, and New York to evaluate the ecological impact of MON 863 *Bt* corn, grown under different insecticide regimes, on abundance of non-target organisms relative to non-transgenic corn. The submitted summary of preliminary findings shows some possible effects of MON 863 on corn field insects. Final reports of these studies are required to be submitted to the Agency for review.

*Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.

II. Soil Fate

Soil organisms may be exposed to Cry3Bb1 protein by exposure to roots, incorporation of aboveground plant tissues into soil after harvest, or by pollen deposited on the soil. Root exposure may occur by

feeding on living or dead roots or, theoretically, by ingestion or absorption after secretion of the Cry protein into the soil. In addition, some evidence suggests that Cry proteins, while bound to some soil components (e.g. clays and humic acids), are recalcitrant to degradation by soil microorganisms but without eliminating their insect toxicity. Several factors influence either the affinity of binding or the rate of degradation. In particular, a neutral pH generally substantially increases degradation. Corn does not grow well below ~pH 5.6; therefore, most corn-growing soils are expected to be at a higher pH. Under most production conditions, corn would not be grown in soils that would inhibit the rate of degradation compared to what is seen at near neutral pH. Nevertheless, these issues are being evaluated on a case-by-case basis by environmental fate studies designed to determine the rate of Cry protein degradation over sufficiently long periods to assure an accurate assessment of degradation in agricultural soils.

MRID No. 451568-04

This study was conducted in accordance with Good Laboratory Practice Standards (40 CFR Part 160).

Methods:

An insect bioassay and an ELISA were conducted to measure the level of functional and non-functional Cry3Bb1 protein present in field soil samples. The amount of lyophilized corn tissue added to the field soil in this study was based on the amount of plant tissue that could potentially be incorporated into the top six inches of soil under field conditions. Since field incorporation of plant tissue usually will not take place until the fall, this amount of Cry3Bb1 protein represents the worst-case scenario during the growing season, including possible exudation of Cry protein through the roots into soil. Based on this calculation, 0.03 g (rounded up from 0.028 g) dry weight plant tissue was added to each gram of dry sandy loam field soil (from Fayette County of Lexington, Kentucky); therefore, 3% of the dry weight of soil was dry weight of lyophilized plant tissue. An additional test was conducted with 10% of the soil containing lyophilized dry weight plant tissue (0.10 g leaf tissue/g soil). Insect bioassays included a mixture of test and control substances with an agar-based insect diet added to wells of bioassay trays. Colorado potato beetle (CPB; *Leptinotarsa decemlineata*) larvae were added 1 larva/well, and each treatment bioassay was replicated twice for a total of 16 CPB/replicate. CPB are more sensitive to the Cry3Bb1 protein then CRW and were, therefore, expected to result in a more measurable response than CRW, the target species. In addition to an insect bioassay, an ELISA was conducted to measure the level of Cry3Bb1 protein present in samples. The ELISA test will only show extractable protein and does not distinguish between functional and non-functional proteins.

Results:

Results from this study show the DT_{50} and DT_{90} (degradation time) for Cry3Bb1 in leaf tissues in sandy loam soil based on the ELISA test to be 2.76 and 9.16 days, respectively. The 21-day ELISA sample was the last to show traces of Cry protein. At 28 days, the Cry3Bb1 protein was below the detection level. The value of these results, however, needs to be considered with regard to biological activity because it is unknown if the extractable protein in the ELISA test was functional or non-functional. Therefore, the insect bioassays were performed with CPB and determined the DT_{50} and DT_{90} to be 2.37 and 7.87 days, respectively. The no-detection level was in the range of the results obtained by ELISA.

Conclusions:
Based on these results, Cry3Bb1 protein does degrade rapidly and does not accumulate in sandy loam soil; however, corn is not necessarily grown in sandy loam soil in all regions. Corn is grown in other soil types, such as clay loam and silt loam soils in various regions of the U.S. Testing clay soils would be considered a "worst-case scenario." In addition, this test does not account for all plant tissue, such as roots, or root exudation of the Cry protein in the field. It is possible that root tissue is degrading slower then leaf tissue in the soil, which may result in a longer duration of degradation time of the Cry3Bb1 protein. Therefore, it was recommended that field testing should be continued in a variety of soil types, including clay and humic acid soils, over a three years to determine the long-term degradation rate and accumulation/persistence of Cry3Bb1 protein in soil.

Since the submission and review of this study (MRID No. 451568-04), the Agency convened an SAP on August 27, 2002 to address corn rootworm PIP non-target issues, including the degradation of *Bt* protein in soil. The aerobic soil degradation study (MRID No. 457571-02) below was designed to address the deficiencies identified in MRID No. 451568-04 and comments made by both the August 27, 2002 SAP and the public.

MRID No. 457571-02
This study is intended to address the issues raised in MRID No. 451568-04:

> "Supplemental to conducting the study in the field with all plant tissue incorporated into the soil in fields that have had MON 863 corn grown for one to three consecutive years. Studies should also be conducted in a variety of soil types particularly soil high in clay and humic acids."

In addition, the previously submitted aerobic soil degradation study (MRID No. 451568-04) utilized plant tissue from Cry3Bb1 transformation event MON 859 rather than MON 863. The study described in the paragraphs that follow involved use of MON 863 corn root and shoot tissue.

Methods:
The test substance consisted of finely ground and lyophilized root and shoot tissue of MON 863 containing the Cry3Bb1 insecticidal protein. The concentration of Cry3Bb1 protein in the lyophilized corn tissue, determined by ELISA, was 487 µg/g in the root and 468 µg/g in the shoot. A purified Cry3Bb1 protein, obtained from a genetically modified *E. coli* strain containing a sequence identical to MON 863 corn, was also included in the study. MON 863 corn shoot and root tissues were collected from field grown plants in Richland, Iowa. The test matrix consisted of three soils collected from the top six inches, Horizon A, of corn fields in Carlyle, Illinois; Monmouth, Illinois; and Richland, Iowa. Soil properties for these three soils (Table 3) and a microbial analysis were characterized. Soil viability was confirmed at test initiation and at approximately four and eight weeks of incubation. Soils were shown to remain active and viable throughout the study (144–228 microbial biomass carbon/50 g soil). The highest concentration of Cry3Bb1 protein in roots 35 days after planting is 66 µg/g. On this basis, the maximum field loading of Cry3Bb1 in corn shoots is 3.93 µg/g soil (equivalent to 8 mg shoots/g soil);

for corn root tissue, the loading is 2.79 µg/g soil (equivalent to 6 mg of roots/g soil). For additional conservatism, the maximum values were exaggerated 3x for dosing of soils. Therefore, 24 mg of shoot tissue and 18 mg of root tissue was added to soils. Also, the purified Cry3Bb1 control dosing concentration was exaggerated ~25x to model the unlikely scenario that large amounts of protein would be exuded by roots into the soil throughout the growing season. Approximately 48 µg of purified protein was added to 0.5 g of soil. Soils from the three test locations were air dried and dosed with the Cry3Bb1 test substance at these rates. All vials were mixed thoroughly and soil moisture adjusted with deionized water to obtain soil moisture of 75% field capacity at 0.33 bar.

Table 3. Physicochemical Characteristics of Soils Collected from Horizon A (Top 6 Inches).

Parameter		***Soil Source***		
		Carlyle, IL	**Monmouth, IL**	**Richland, IA**
USDA Textural Class		Silt Loam	Silt Loam	Silt Loam
Particle Size Distribution (%)	Sand Silt Clay	21 58 21	18 56 26	13 62 25
Bulk Density (g/cm^3)		1.00	1.03	1.07
% Organic Matter		2.5	4.6	4.0
Cation Exchange Capacity (meq/100 g)		16.6	23.9	20.7
Field Moisture Capacity	@ 1/3 Bar @ 15 Bar	28.2 15.7	30.7 17.9	30.3 17.2

*Table taken from page 29 of MRID No. 457571-02.

Results:

Cry3Bb1 protein levels in soil sample extracts determined by ELISA show that Cry3Bb1 protein concentrations were near or below the ELISA detection limit (LOQ) of 0.16 µg/g after 2 months of incubation. After two months of soil incubation, Cry3Bb1 concentrations were at least an order of magnitude below the initial concentration of Cry3Bb1 in the dosed samples. The DT_{50} values for all dosing regimes and soil types ranged from 0.6 days to 2.3 days, and the DT_{90} values ranged from 4.03 days to 50 days (Table 4). Purified Cry3Bb1 protein degraded faster than when corn shoot or root tissue was applied. Visual observation verified that root tissues are slower to degrade in soil than shoot tissue.

These results also indicate, as expected, that the longer DT_{50} and DT_{90} values for corn tissues are due to the time required for the tissue to decay and for the Cry3Bb1 protein to move from tissue to soil. In addition, the rapid degradation (DT_{90} of 4.0 to 5.2 days) of the purified Cry3Bb1 protein suggests that any Cry3Bb1 protein reaching the soil by root exudation or release from slowly decaying plant tissue would be >90% degraded in less than 6 days.

Table 4. DT_{50} and DT_{90} Estimates for the Dissipation of Cry3Bb1 Protein in Soils.

Protein Source	Soil Source	DT_{50}[a] (Days)	DT_{90}[b] (Days)
Purified Protein	Carlyle, IL	**0.63** (0.49, 0.78)[c]	**4.03** (3.67, 4.42)
	Monmouth, IL	**0.64** (0.50, 0.80)	**4.18** (3.77, 4.62)
	Richland, IA	**0.73** (0.50, 1.00)	**5.23** (4.48, 6.07
MON 863 Corn Root Tissue	Carlyle, IL	**1.74** (0.78, 3.20)	**27.29** (14.52, 50.58)
	Monmouth, IL	**1.19** (0.86, 1.57)	**12.48** (9.90, 15.67)
	Richland, IA	**2.27** (1.68, 2.98)	**50.02** (34.57, 72.18)
MON 863 Corn Shoot Tissue	Carlyle, IL	**1.45** (0.82, 2.30)	**18.68** (12.61, 27.46)
	Monmouth, IL	**0.90** (0.61, 1.24)	**7.43** (6.01, 9.12)
	Richland, IA	**1.77** (1.40, 2.21)	**28.59** (23.16, 35.23)

[a] DT_{50} = Time to 50% dissipation of original protein concentration
[b] DT_{90} = Time to 90% dissipation of original protein concentration
[c] Lower and upper 95% confidence interval on the DT value
*Table taken from page 36 of MRID No. 457571-02.

Conclusions:

It is difficult to determine a DT_{50} or DT_{90} for Cry3Bb1 expressed in corn tissue in the field from this study because corn shoot and root tissue were analyzed separately and not all plant material was included. Therefore, methods utilized in this study do not represent actual field conditions. It is unknown whether these laboratory results can be adequately correlated to the field. Additional field studies should be conducted that include the incorporation of all non-harvested plant tissue in a variety of soil types, particularly areas high in clay (>26% tested here) and humic acids. These studies should be conducted for at least one growing season after harvest and continue until no Cry3Bb1 protein is detected. In addition, the persistence of the Cry3Bb1 protein under less than optimum conditions (e.g., high or low temperatures; high or low soil moisture content) should be examined. Additional studies, conducted to address the degradation of Cry3Bb1 protein in the soil, should include an insect bioassay utilizing a known sensitive species (e.g., Colorado potato beetle).

These conclusions are based in part on the August 27, 2002 SAP and several public comments. The Panel concluded that several different soils should be examined and monitored for a minimum of one growing season after harvest and continued until the Cry3Bb1 protein can no longer be detected. The

Panel also recommended that an additional sample or two should be examined to verify that an analytical error was not the cause for the lack of detection. According to the Panel, at least two additional soil types should be evaluated for Cry3Bb1 persistence. Soils that are high in organic matter and clay should be the focus, since there is the highest potential of persistence in these soil types. Other soils, however, should still be considered. The Panel also recommended that the soil degradation studies be conducted under less than optimum conditions, such as high or low temperatures or high or low moisture content. Since corn roots grow deep into the soil to areas with reduced microbial activity, degradation rates may be reduced. Therefore, degradation of Cry3Bb1 from deep sites should also be examined. The Panel also addressed the protein source that is appropriate for the soil degradation studies. Future studies should utilize plant material that is representative of actual field conditions. For example, whole plant tissue should be incorporated. Plant tissue should not be ground prior to incorporation because it artificially increases the surface area exposed to microorganisms, which may then lead to an increase in the rate of degradation of the protein. Since more protein may be present than is detected by an ELISA, an insect bioassay, using a sensitive species such as the Colorado potato beetle, should be conducted. The SAP concluded that "[r]eal life or true persistence is likely to be equal to or less than that measured with ELISA." If an ELISA is conducted, the results should be compared to results from a beetle bioassay.

This study, although rated as supplemental, provides further evidence that the Cry3Bb1 protein in MON 863 produces no short-term risk of unreasonable adverse effects for the environment.

*Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.

III. Effects on Soil Microorganisms

Numerous published studies indicate that exposure to Cry protein produced in *Bt* PIP crop plants does not adversely affect soil microorganisms (Sanvido *et al*. 2007; Oliveira *et al*. 2008). In addition, *Bt* toxin released from root exudates and biomass of *Bt* corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil (Saxena and Stotzky 2001). Other research findings conclude no *Bt*-related risks have evolved from the decomposition of *Bt*-corn leaves for the meso- and macrofauna soil community (Hönemann *et al*. 2008). Although a minimal transient increase and shift in microbial populations may result from the presence of transgenic plant tissue in soil, no adverse effects have been attributed to the Cry protein.

In addition, there are several ongoing U.S. Department of Agriculture and EPA Office of Research and Development funded research projects evaluating the effects of *Bt* crops on soil microbial flora. If adverse effects are seen from this or any other research, the Agency will take appropriate action to mitigate potential risks.

With regard to the impact of genetically engineered crops on soil, it is important to note that agricultural practices themselves cause large changes in soil and soil microbial composition. Furthermore, factors

such as variations in seasons and weather, plant growth stage, and plant varieties, independent of being genetically engineered, are also responsible for significant shifts in soil microbial communities. To date, most studies with genetically engineered crops have shown minor or no effects on soil microbes beyond the variation caused by the factors listed above.

IV. Horizontal Transfer of Transgenes from Bt Crops to Soil Organisms

EPA has evaluated the potential for horizontal gene transfer (HGT) from *Bt* crops to soil organisms and has considered possible risk implications if such a transfer were to occur. Genes that have been engineered into *Bt* crops are mostly found in, or have their origin in, soil-inhabiting bacteria. Soil is also the habitat of anthrax, tetanus, and botulinum toxin-producing bacteria. Transfer of these genes and/or toxins to other microorganisms or plants has not been detected. Furthermore, several experiments (published in scientific journals), that were conducted to assess the likelihood of HGT, have been unable to detect gene transfer under typical environmental conditions. Horizontal gene transfer to soil organisms has only been detected with very promiscuous microbes under laboratory conditions designed to favor transfer.

As a result of these findings, which suggest that HGT is at most an artificial event, and the fact that the *Bt* toxins engineered into Cry3Bb1 corn are derived from soil-inhabiting bacteria, EPA has concluded that there is a low probability of risk from HGT of transgenes found in Cry3Bb1-producing corn.

V. Gene Flow and Weediness Potential

The movement of transgenes from the host plant into weeds has been a significant concern for the Agency due to the possibility of novel exposures to the pesticidal substance. The Agency has determined that there is no significant risk of gene capture and expression of Cry3Bb1 protein by wild or weedy relatives of corn in the U.S., its possessions, and/or its territories. In addition, the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has made this same determination under its statutory authority under the Plant Pest Act.

Under FIFRA, the Agency has reviewed the potential for gene capture and expression of *Bt* endotoxins by wild or weedy relatives of corn, cotton, and potatoes in the U.S., its possessions, and/or its territories. *Bt* plant-incorporated protectants that have been registered to date have been expressed in agronomic plant species that, for the most part, do not have a reasonable possibility of passing their traits to wild native plants. Feral species related to these crops, as found within the United States, cannot be pollinated by these crops (corn, potato, and cotton) due to differences in chromosome number, phenology (i.e., periodicity or timing of events within an organism's life cycle as related to climate, e.g., flowering time) and habitat. The only exception, however, is the possibility of gene transfer from *Bt* cotton to wild or feral cotton relatives in Hawaii, Florida, and the Caribbean.

The Scientific Advisory Panel meeting held on October 18–20, 2000 further discussed the matter of gene flow and offered some issues for consideration in this matter. The panel agreed that the potential

for gene transfer between corn (maize) and any receptive plants within the U.S., its possessions, and/or its territories was of limited probability and nearly risk free.

Concern over the potential for species related to maize (*Zea mays* ssp. *mays*), such as *Tripsacum* species and the teosintes, as potential recipients of gene flow from genetically modified *Zea mays* indicated a need for review of what is known related to gene flow potential of *Z. mays*. Some *Zea* species, such as the teosintes, are known to be interfertile with maize and are discussed as potential recipients of pollen-directed gene flow from maize. This issue is of particular concern based upon the increased planting of genetically modified maize. Therefore, the Agency conducted a reevaluation in early 2000, the results of which are reported here.

a. *Zea mays* ssp. *mays* - Maize - General Biology

Zea mays is a wind-pollinated, monoecious, annual species with imperfect flowers. This means that spatially separate tassels (male flowers) and silks (female flowers) are found on the same plant, a feature that limits inbreeding. A large variety of types are known to exist (e.g., dent, flint, flour, pop, sweet) and have been selected for specific seed characteristics through standard breeding techniques. Maize cultivars and landraces are known to be diploid (2n = 20) and interfertile to a large degree. However, some evidence for genetic incompatibility exists within the species (e.g., popcorn x dent crosses; Mexican maize landraces x Chalco teosinte). *Zea mays* has been domesticated for its current use by selection of key agronomic characters, such as a non-shattering rachis, grain yield, and resistance to pests. The origin of corn is thought to be in Mexico or Central America, based largely on archaeological evidence of early cob-like maize in indigenous cultures approximately 7,200 years ago.

A recent study has indicated that cross-pollination of commercial maize cultivars at 100 feet downwind from the source of genetically modified maize was 1%, and this proportion declined exponentially to 0.1% at 130 feet and further declined to 0.03% at 160 feet. At 1,000 feet, the farthest distance measured, no cross-pollination was detected (Jemison and Vayda 2000). For production of Foundation Seed, a distance of 660 feet has been generally required to mitigate outcrossing between different genotypes. The relatively large size of corn pollen and its short viability period under most conditions reduce long distance transfer for purposes of outcrossing (Schoper, personal communication,1999). Under conditions of high temperature or low humidity, corn pollen may only survive for a matter of minutes. Under more favorable conditions in the field or with controlled handling in the laboratory, pollen life may be extended to several hours.

b. *Tripsacum* species - Gama Grass - General Biology

Close relatives of corn or maize are found in the genus *Tripsacum*. Sixteen species of *Tripsacum* are known worldwide and generally recognized by taxonomists and agrostologists; most of the 16 different *Tripsacum* species recognized are native to Mexico, Central America, and South America, but three occur within the U.S. Hitchcock (1971) reports the presence of three species of *Tripsacum* in the continental United States: *Tripsacum dactyloides*, *Tripsacum floridanum*, and *Tripsacum lanceolatum*.

Of these, *T. dactyloides*, Eastern Gama Grass, is the only species of widespread occurrence and of any agricultural importance. It is commonly grown as a forage grass and has been the subject of some agronomic improvement (i.e., selection and classical breeding). *T. floridanum* is known from southern Florida, and *T. lanceolatum* is present in the Mule Mountains of Arizona and possibly southern New Mexico.

For the species occurring in the United States, *T. floridanum* has a diploid chromosome number of 2n = 36 and is native to Southern Florida; *T. dactyloides* includes 2n = 36 forms, which are native to the central and western U.S., and 2n = 72 forms, which extend along the Eastern seaboard and along the Gulf Coast from Florida to Texas but which have also been found in Illinois and Kansas; these latter forms may represent tetraploids (x = 9 or 18)(Lambert, personal communication, 1999); and *T. lanceolatum* (2n = 72), which occurs in the southwestern U.S. *Tripsacum* differs from corn in many respects, including chromosome number (*T. dactyloides* n = 18; *Z. mays* n = 10). Many species of *Tripsacum* can cross with *Zea*, or at least some accessions of each species can cross, but only with difficulty and the resulting hybrids are primarily male and female sterile (Duvick, personal communication, 1999; Galinat 1988; Wilkes 1967). *Tripsacum*/maize hybrids have not been observed in the field but have been accomplished in the laboratory using special techniques under highly controlled conditions.

Eastern Gama Grass is considered by some to be an ancestor of *Z. mays* or cultivated maize (Mangelsdorf 1947), while others dispute this (Galinat 1983; Iltis 1983; Beadle 1980), based largely on the disparity in chromosome number between the two species (maize n = 10; Gama Grass x = 9 or 18, with diploid, triploid, and tetraploid races existing; 2n = 36 or 72), as well as radically different phenotypic appearance. Albeit with some difficulty, hybrids between the two species have been made (Mangelsdorf and Reeves 1939; DeWald, personal communication,1999). In most cases, these progeny have been sterile or viable only by culturing with *in vitro* "embryo rescue" techniques.

Even though some *Tripsacum* species occur in areas where maize is cultivated, gene introgression from maize under natural conditions is highly unlikely, if not impossible (Beadle 1980). Hybrids of *Tripsacum* species with *Z. mays* are difficult to obtain outside of the controlled conditions of laboratory and greenhouse. Seed obtained from such crosses are often sterile or progeny have greatly reduced fertility. Approximately 10–20% of maize-*Tripsacum* hybrids will set seed when backcrossed to maize, and none are able to withstand even the mildest winters. The only known case of a naturally occurring *Zea - Tripsacum* hybrid is a species native to Guatemala known as *Tripsacum andersonii*. It is 100% male and nearly 99% female sterile and is thought to have arisen from gene flow to teosinte, but the lineage is uncertain (Doebley, personal communication, 2000). *Z. mays* is not known to harbor properties that indicate it has weedy potential and, other than occasional volunteer plants in the previous season's corn field, maize is not considered as a weed in the U.S.

In a telephone conversation with Dr. Chester "Chet" DeWald (Agricultural Research Service of the USDA; Woodward, Oklahoma), a geneticist working on improvement of grasses, he stated that relatively few accessions of *T. dactyloides* will cross with maize, and the majority of progeny are not fertile or viable even in those that do. In controlled crosses, if the female parent is maize, there is a

greater likelihood of obtaining viable seed. When these hybrids have been backcrossed to maize in attempts to introgress *Tripsacum* genes for quality enhancement or disease resistance, the *Tripsacum* chromosomes are typically lost in successive generations. In many instances where hybridization has been directed between these two species, the resultant genome is lacking in most or all of the chromosomal complements of one of the parent species in subsequent generations.

Only recently has Dr. DeWald (or anyone else) succeeded in obtaining a true *Tripsacum* cytoplasm with a maize nuclear background. This was done by using gama grass as the female parent and maize as the male or pollen donor. Numerous accessions were tested and crosses made before this came to fruition. The *Tripsacum*-derived mitochondrial chondrome and chloroplast plastome in these hybrids contribute to the seed qualities of the plants, but the nuclear genome appears to be totally maize in origin (DeWald *et al*. 1999).

Dr. DeWald concluded that the possibility of maize contributing genetic material to Eastern Gama Grass through random pollen flow in agricultural or natural situations is extremely remote based upon his experience trying to create hybrids under the best of conditions. He also felt that no other known grass species present in the continental U.S. would interbreed with commercial maize populations (i.e., be recipients of pollen-directed gene flow). This is in agreement with Holm *et al*. (1979), who determined that none of the sexually compatible relatives of corn in the U.S. are considered serious, principal, or common weeds in the U.S.

c. *Zea* species - Teosintes - General Biology

Teosintes—specifically *Zea mays* ssp. *mexicana* (Schrader) Iltis, *Zea mays* ssp. *parviglumis* Iltis and Doebley, *Zea mays* ssp. *huehuetenangensis* (Iltis and Doebley) Doebley, *Zea luxurians* (Durieu and Ascherson) Bird, *Zea perennis* (Hitchc.) Reeves and Mangelsdorf, and *Zea diploperennis* Iltis, Doebley and Guzman—have co-existed and co-evolved in close proximity to maize in the Americas over thousands of years; however, maize and teosinte maintain distinct genetic constitutions despite sporadic introgression (Doebley 1990).

The teosintes retain a reduced cob-like fruit/inflorescence that shatters more than cultivated maize but still restricts the movement of seeds as compared to more widely dispersed weedy species. Hence, the dispersal of large numbers of seeds, as is typical of weeds, is not characteristic of teosintes or maize. In their native habitat, some teosintes have been observed to be spread by animals feeding on the plants. Teosintes and teosinte-maize hybrids do not survive even mild winters and could not propagate in the U.S. Corn Belt. Additionally, some types have strict day length requirements that preclude flowering within a normal season (i.e., they would be induced to flower in November or December) and, hence, seed production under our temperate climate (Beadle 1980; Iltis, personal communication, 2000; Wilkes, personal communication, 2000; Wilkes 1967).

Since both teosinte and *Tripsacum* are included in botanical gardens in the U.S., the possibility exists (although unlikely) that exchange of genes could occur between corn and its wild relatives. The Agency

is not aware, however, of any such case being reported in the United States. Gene exchange between cultivated corn and transformed corn would be similar to what naturally occurs at the present time within cultivated corn hybrids and landraces. Plant architecture and reproductive capacity of the intercrossed plants will be similar to normal corn, and the chance that a weedy type of corn will result from gene flow with cultivated corn is extremely remote.

Like corn, *Z. mays* ssp. *mexicana* (annual teosinte) and *Z. diploperennis* (diploid perennial teosinte) have 10 pairs of chromosomes, are wind pollinated, and tend to outcross but are highly variable species that are often genetically compatible and interfertile with corn, especially when maize acts as the female parent. *Z. perennis* (perennial teosinte) has 20 pairs of chromosomes and forms less stable hybrids with maize (Edwards *et al*. 1996; Magoja and Pischedda 1994). Corn and compatible species of teosinte are capable of hybridization when in proximity to each other. In Mexico and Guatemala, teosintes exist as weeds around the margins of corn fields. The F_1 hybrids have been found to vary in their fertility and vigor. Those that are fertile are capable of backcrossing to corn. A few isolated populations of annual and perennial teosinte were said to exist in Florida and Texas, respectively (USDA APHIS 1997). The Florida populations were presumably an escape from previous use of *Z. mays* ssp. *mexicana* as a forage grass, but local botanists have not documented any natural populations of this species for approximately twenty-five years (Bradley, personal communication, 2000; Hall, personal communication, 2000; Wunderlin, personal communication, 2000).

Consultation with botanists and agronomists familiar with Texas flora suggested that no teosinte populations exist in the state (Benz, personal communication, 2000; Read, personal communication, 2000; Orzell, personal communication, 2000; Wilson, personal communication, 2000). Further, given the day length characteristics of *Z. diploperennis*, it is highly unlikely a sustaining population would result from introduction of this species. *Z. mays* ssp. *mexicana*, *Z. mays* ssp. *parviglumis*, *Z. luxurians*, and *Z. diploperennis* may cross with maize to produce fertile hybrids in many instances (Wilkes 1967). None of these teosinte species have, however, been shown to be aggressive weeds in their native or introduced habitats (Schoper, personal communication, 1999). Except for special plantings as noted above, teosinte is not present in the U.S. or its territories. Its natural distribution is limited to Mexico, Honduras, Nicaragua, El Salvador, and Guatemala.

Given the cultural and biological relationships of various teosinte species and cultivated maize over the previous two millennia, it would appear that significant gene exchange has occurred (based upon morphological characters) between these two groups of plants, and that no weedy types have successfully evolved as a result. More recent cytogenetic, biochemical, and molecular analyses have indicated that the degree of gene exchange is far less than previously thought (Doebley 1984; Doebley *et al*. 1987; Kato 1997a; Kato 1997b; Smith *et al*. 1985). Partial and complete gametophytic incompatibility has been documented among cultivated maize, landraces, and teosinte (Kermicle 1997). The former is demonstrated by differential pollen growth and a skewed recovery of alleles linked to incompatibility genes. Complete incompatibility mechanisms serve to isolate a species or subspecies and are evidenced as pollen exclusion or non-functioning of pollen types on certain genotypes. Attempts to cross six collections of Z. mays ssp. *mexicana* with U.S. maize cultivars (W22, W23) yielded no or few seeds in five of the six groups (Kermicle and Allen 1990).

Based on the ability of maize to hybridize with some teosintes, the suggestion of previous genetic exchange amongst these species over centuries, and their general growth habits, any introgression of genes into wild teosinte from *Z. mays* is not considered a significant agricultural or environmental risk. The growth habits of teosintes are such that the potential for serious weedy propagation and development is not biologically plausible in the United States.

d. Conclusion

The potential for pollen-directed gene flow from maize to Eastern Gama Grass is extremely remote. This is evidenced by the difficulty with which *T. dactyloides* x *Z. mays* hybrids are produced in structured breeding programs. Additionally, the genus does not represent any species considered as serious or pernicious weeds in the United States or its territories. Any introgression of genes into this species as a result of cross fertilization with genetically modified maize is not expected to result in a species that is weedy or difficult to control. In many instances where hybridization has been directed between these two species, the resultant genome is lacking in most or all of the maize chromosomal complement in subsequent generations.

Many of the *Zea* species loosely referred to as "teosintes" will produce viable offspring when crossed with *Z. mays* ssp. *mays*. None of these plants are known to harbor weedy characteristics and none of the native teosinte species, subspecies, or races are considered aggressive weeds in their native or introduced habitats. In fact, many are on the brink of extinction where they are indigenous and will be lost without human intervention (i.e., conservation measures). Further, none of the landraces or cultivated lines of *Z. mays* are considered to have weedy potential and are generally considered incapable of survival in the wild as a result of breeding practices (i.e., selection) during domestication of the crop.

iii. Endangered Species Considerations

Cry3 proteins, including Cry3Bb1, are known to be highly specific against coleopteran insects and are not hazardous to vertebrate animals. It has been generally demonstrated that Cry3 proteins do not pose a hazard to non-target animals or invertebrates. The Cry3Bb1 protein appears to be specifically toxic to Chrysomelid beetles, including corn rootworm (*Diabrotica* spp.) and Colorado potato beetle (*Leptinotarsa decemlineata*) (MRID No. 455328-07). Currently, there are no Chrysomelid species listed on the endangered species list, and no other species are known to be sensitive to Cry3Bb1. Therefore, no adverse effects from Cry3Bb1 (Event MON 863) are expected against endangered species. Nevertheless, all endangered/threatened beetle species habitats, found in the counties where corn is grown, were examined to determine possible exposure to corn pollen. Their habitat (and breeding grounds) was found not to overlap with corn fields. Endangered beetles will not be exposed to potentially harmful levels of corn tissue or pollen containing Cry3Bb1 protein.

Terrestrial and aquatic exposure were considered in this assessment since non-target coleopterans may be exposed to the Cry3Bb1 protein within corn fields or in surrounding areas from plant tissue (e.g., pollen) movement offsite. The distance pollen moves outside of the corn field must be considered. Published data show that less than 25 grains of pollen per square centimeter are expected 4–5 meters from the corn field edge. A relative comparison of surface ratio of milkweed to other substrates (e.g., other host plants, arthropod prey, and animal carrion) can be used as a basis for estimating the amount of pollen that may leave the field. The maximum concentration of Cry3Bb1 protein has been determined to be 93 µg/g fresh weight pollen. Based on this concentration, <0.03 µg Cry3Bb1 protein/g of diet would be expected to be deposited 4–5 meters from the field edge. The potential for aquatic organisms to be exposed to the Cry3Bb1 protein is minimal. Such exposure would occur from runoff of the protein (either free or sequestered in plant debris) into adjacent water bodies or pollen drift. Since movement of Cry3Bb1 in soil into water bodies is expected to be negligible, pollen drift was considered the primary source of potential hazard to endangered aquatic Coleoptera. According to estimates based on published studies, if 100% of the pollen grains leaving the field were deposited in a 1 hectare pond with 2 meters depth and located ≥1 mile from the edge of the corn field, <0.0001 µg Cry3Bb1/mL of water would be expected. This is a few orders below the toxic level to any insect.

Many of the endangered and threatened beetles occur in cave or aquatic habitats. None of these endangered beetles are expected to occur in or near corn fields. The American burying beetle may occur in old fields or cropland hedge rows. Based upon the feeding habits of the American burying beetle, however, it is not expected to occur within corn fields or be exposed to Cry3Bb1 protein. Adult American burying beetles are classified as opportunistic scavengers that feed on anything dead and bury vertebrate carcasses on which their larvae feed. Carrion regurgitated by adults is fed to larvae until they are able to feed directly on a carcass.

In addition, Monsanto conducted a hazard assessment, exposure assessment, and risk characterization to demonstrate that Cry3Bb1 does not pose a risk to endangered Coleoptera (MRID No. 455770-03). This endangered species assessment was based on the Hazard Evaluation Division, Standard Evaluation Procedure – Ecological Risk Assessment (U.S. EPA 1986). The Agency reviewed this assessment and found it acceptable.

An examination of the endangered bird and bat species shows that their breeding habitats are mostly non-agricultural. Insectivorous bats do not prey on larvae; they rely on flying insects. Taking these, and other pertinent issues into consideration, it becomes apparent that reduction in the target pests of corn would not have an effect on the food source of endangered birds and bats. Of those that do encroach on agricultural fields and in the rare instances where these species may feed on the target pests, the reduction in the pest species will merely cause them to rely on other plentiful insects as a source of food. Submitted and published field data show that a wide variety of insects remain abundant in Cry3Bb1 corn fields, as opposed to non-*Bt* fields when conventional insect pest control practices are used. Therefore, the data show that *Bt* crops should actually be beneficial to bird and bat populations.

The reviewed non-target data confirm the expectation that Cry3Bb1 corn will have no effects on endangered and/or threatened species listed by the U.S. Fish and Wildlife Service, including mammals,

birds, or terrestrial and aquatic plants and invertebrate species. Therefore, no consultation with the U.S. Fish and Wildlife Service is required under the Endangered Species Act.

iv. Environmental Assessment Summary

The Agency is using a MHD tiered system for biopesticide non-target wildlife hazard assessment. When no adverse effects at the maximum hazard dose are observed, the Agency concludes that there are no unreasonable adverse effects from the use of the pesticide. From all of the required and voluntarily developed indicator and host range species test data on Cry3Bb1 corn, including the supplementary two-year field data, the Agency concludes that the levels of Cry3Bb1 protein in corn will not pose unreasonable adverse effects to corn field flora and fauna. Available data also indicate that there should be minimal short-term accumulation of Cry3Bb1 protein in agricultural soil. In addition, no effects on listed endangered and threatened species are expected from the proposed Cry3Bb1 CRW-resistant corn registration.

At present, the Agency is aware of no identified significant adverse effects of Cry3Bb1 proteins on the abundance of non-target beneficial organisms in any population in the field, whether they are pest parasites, pest predators, or pollinators. Field testing and field census data submitted to the Agency show minimal to undetectable changes in the beneficial insect abundance or diversity. In corn fields, densities of predatory and non-target insects are generally higher on Cry3Bb1 corn than non-*Bt* corn, primarily because the Cry3Bb1 corn is not subjected to the same number of applications of nonspecific pesticides. Two-year invertebrate abundance studies do not show a shift in the biodiversity in Cry3Bb1 corn, except in cases where the predators are dependent on the pest insect as prey. In contrast, treatment with chemical pesticides, when studied, had significant effects on the total numbers of insects and on the numbers within the specific groups. To date, the available field test data show that, compared to crops treated with conventional chemical pesticides, the transgenic crops have no detrimental effect on the abundance of non-target insect populations. Despite these conclusions, annual insect monitoring of representative commercial fields will continue for long-term biodiversity effects assessment.

The Agency believes that cultivation of Cry3Bb1 corn may result in fewer adverse impacts to non-target organisms than result from the use of chemical pesticides. Under normal circumstances, Cry3Bb1 corn requires substantially fewer applications of chemical pesticides. This should result in fewer adverse impacts to non-target organisms because application of nonspecific conventional chemical pesticides is known to have an adverse effect on non-target beneficial organisms found living in the complex environment of an agricultural field. Many of these beneficial organisms are important integrated pest management controls for secondary pests, such as aphids and leafhoppers. The overall result of cultivation of corn expressing Cry3Bb1 proteins is that the number of chemical insecticide applications for non-target pest control is reduced for management of multiple pest problems.

The movement of transgenes from Cry3Bb1 host plant into weeds and other crops has also been considered. The Agency has determined that there is no significant risk of gene capture and expression of Cry3Bb1 protein by wild or weedy relatives of corn in the U.S., its possessions, and/or territories. The

fate of Cry3Bb1 protein in soils and indirect effects on soil biota has also been evaluated. Test data show that most of the Cry protein deposited into soil is quickly degraded, although a residual amount may persist in biologically active form for a much longer period of time. It is also reported that the same degree of *Bt* Cry protein persistence takes place in soils that have been exposed to repeat *Bt* spray applications when compared to soil exposed to growing *Bt* crop. Limited data do not indicate that Cry proteins have any measurable effect on microbial populations in the soil. Horizontal transfer from transgenic plants to soil bacteria has not been demonstrated. Published studies of *Bt* Cry protein in soil show no effect on bacteria, actinomyces, fungi, protozoa, algae, nematodes, springtails, or earthworms. In addition, new plants planted in *Bt* Cry protein-containing soil do not take up the *Bt* protein.

This assessment finds no hazard to the environment, at the present time, from cultivation of corn expressing Cry3Bb1 protein.

v. Supplemental Studies Needed for Long-Term Cry3Bb1 Non-Target Hazard Assessment

The Agency has sufficient information to believe that there is no risk from the proposed uses of Cry3Bb1 corn to non-target wildlife, aquatic, and soil organisms. Nonetheless, after consultation with the FIFRA Scientific Advisory Panel in August, 2002 and in response to several public comments, the Agency is requesting additional data, which could provide more weight to support the Agency's conclusions. Specifically, the Agency is requesting the data, as set forth in Table 5, to ascertain any possible adverse environmental effects from long-term use of Cry3Bb1 and to test on more appropriate non-target invertebrates found in corn fields. The Agency does not believe that these data requirements were reasonably foreseeable by Monsanto at the time of its application.

Table 5. Supplemental Data Requirements.

Testing Category	Type of Data
Avian chronic exposure testing	The submitted avian dietary toxicity data are not sufficient to make a final avian hazard assessment from repeated exposure(s) to higher doses of Cry3Bb1 corn. A six-week broiler dietary study with 60%–70% MON 863 corn in the diet is needed to assess hazard to wild and domesticated fowl from chronic exposure to high levels of Cry3Bb1 protein.
Non-target insect more appropriate for corn fields	Conduct a maximum hazard dose laboratory toxicity test with *Orius insidiosus* (minute pirate bug).
Non-target insect more appropriate for corn fields	Conduct a maximum hazard dose laboratory toxicity test with a carabid (ground beetle).
Non-target insect more appropriate for corn fields	Maximum hazard testing with *Tetraopes* (red milkweed beetle) should be performed because they are a more logical choice than the monarch butterfly.
Field community effects	Submit final results to field studies previously summarized in MRID No.456530-03. The carabid and nematode data are of particular interest.
Ecosystem effects	Additional long-range field studies should also be conducted based on recommendations of the August, 2002 SAP.
Soil fate studies	Additional long-range soil degradation field studies should also be conducted, including the parameters outlined by the August, 2002 SAP.

*Note for 2010: There is an update to this summary. See section II(C)(2)(b) ("Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)") of this BRAD.

b. Terms and Conditions of the Corn Event MON 863 Registration (February 2003 – September 2010)

When Corn Event MON 863 was first registered on February 24, 2003, the Agency issued a registration notice to Monsanto that, given the conclusions set forth in the initial environmental risk assessment (see section II(C)(2)(a) of this BRAD), contained the following four requirements for further environmental effects data:

> "Submit field degradation studies evaluating accumulation and persistence of Cry3Bb1 in several different soils in various strata. Representative fields must have been planted with MON 863 and include both conventional tillage and no-till samples and be harvested under typical agronomic conditions. Sampling must continue until the limit of detection is reached. Studies should include soils with high levels of a variety of clays. Both ELISA and insect bioassays need to be conducted and compared to determine if Cry3Bb1 is accumulating or persisting in soil samples…a final report is due two years from the date of registration."

> "Submit laboratory toxicity tests with *Orius insidiosus* (minute pirate bug), carabid (ground beetle), and *Tetraopes* (red milkweed beetle) within 24 months of the date of registration…."
>
> "Full-scale field or semi-field studies with appropriate end points and statistical power must be conducted. Submit intermediate and multi-year non-target organism field studies with statistical power. You must submit final results to field studies previously summarized in MRID No. 456530-03…."
>
> "Submit a six week broiler dietary study with 60%–70% MON 863 corn in the diet that is of appropriate duration to represent the start and growing periods of the test species. Balanced diets should be formulated according to the National Research Council Guidelines ("Nutrient Requirements of Poultry," Ninth Revised Edition, 1994) with the energy requirements of the test species being met by the inclusion of corn in the diet to assess hazards from chronic exposure of wild and domesticated fowl…."

For the Corn Event MON 863 registration, the abovementioned requirements for additional environmental effects data have been satisfied by submission of appropriate studies and a request for a waiver from conducting the laboratory toxicity test with the *Tetraopes*; summaries of this information are presented in Table 6.

Current ecological effects data, to include those conditional data referenced in Table 6, and EPA reviews of Cry3Bb1 protein support the Agency's original determination that adverse effects will not occur to non-target organisms. Due to a demonstrated lack of toxicity and/or exposure, no effects from Cry3Bb1 protein are anticipated for any non-target species, including federally listed threatened and endangered ("listed") lepidopteran and coleopteran species and their designated critical habitats. The Agency is therefore upholding its determination that the registered uses of Cry3Bb1 will have "No Effect," direct or indirect, on endangered or threatened terrestrial or aquatic species as listed by the U.S. Fish and Wildlife Service and the National Marine Fisheries Service.

When the docket for the expiring *Bt* corn registrations was opened for public comment on August 4, 2010 (Docket Number EPA-HQ-OPP-2010-0607), the Agency noted its awareness of a recently published laboratory study showing reduced growth in shredding caddis flies exposed to anti-lepidopteran Cry1A protein corn litter (Rosi-Marshall *et al*. 2007). Given the findings of this particular study, the Agency proposed requiring additional aquatic invertebrate data for the Cry34Ab1 and Cry35Ab1 proteins—either a 7- to 14-day *D. magna* study or a dietary study evaluating the effects of these proteins on an aquatic invertebrate that represents the functional group of a leaf shredder in headwater streams. Since the 2007 Rosi-Marshall *et al*. publication, numerous researchers have published peer-reviewed studies that identify issues with the scientific merit and relevance of the original caddis fly study (Swan *et al*. 2009, Jensen *et al*. 2010, summarized by Beachy *et al*. 2008, Parrott 2008, and Wolt and Peterson 2010). In response to comments received on the proposed terms and conditions for the extension of the 2010 expiring *Bt* corn registrations, the Agency conducted a

literature review of these recently published studies. Criticisms of the Rosi-Marshall *et al*. study included several findings: (1) adverse effects were not caused by toxicity of Cry1A but, rather, by other differences between plant test substances (Jensen *et al*. 2010); (2) the abundance of Trichoptera in streams containing residues of Cry1A was not reduced (Chambers *et al*. 2007); and (3) while post-harvest crop residue was identified as the most likely route of exposure (Carstens *et al*. 2010), aquatic exposure to biotech crops has been shown to be limited temporally and spatially with low to negligible exposure concentrations of Cry proteins in post-harvest crop tissues (Swan *et al*. 2009, Chamber *et al*. 2010, Jensen *et al*. 2010, Wolt and Peterson 2010, Carstens *et al*. 2010). In light of these results, the Agency is not requiring additional aquatic invertebrate studies to assess hazard to aquatic shredder species for existing Cry protein PIP registrations.

Table 6. Environmental Effects Data/Information Submitted in Response to Conditions of Registration for Corn Event MON 863.

Study Title	Summary	MRID No.
Waiver Request from Conducting a Laboratory Toxicity Test with *Tetraopes* (Red Milkweed Beetle)	The Agency notes that further investigation of the biology and life cycle of the red milkweed beetle demonstrates that there will be little or no exposure of larvae under natural conditions. Although adults may be exposed to *Bt* corn pollen while feeding on milkweed leaves, *Bt* is typically less toxic to adults than larvae. Further, there is no protocol for rearing red milkweed beetles in the laboratory, and the development of such a laboratory assay would be difficult due to the red milkweed beetle's long development time. Therefore, on October 1, 2003, the Agency granted Monsanto's request to waive the requirement for conducting a red milkweed beetle study as set forth in the notice of registration (February 24, 2003). **(Unknown reference)**	N/A
Comparison of Broiler Performance When Fed Diets Containing Events MON 863, Parental Line or Commercial Corn	Day-old commercial broiler chickens (Ross x Ross) were fed diets containing either transgenic corn line MON 863 (containing *Bacillus thuringiensis* CRY3BB protein), a non-transgenic parental corn line, or a non-transgenic commercial corn reference line for 42 days. There were no treatment-related, biologically significant differences among groups for mortality; live weight; feed intake or efficiency; carcass weight; fat pad, breast meat, thigh, drum, or wing weights; or breast and thigh moisture, fat, and protein content. **Classification: Acceptable** **(Reviewed in U.S. EPA (2010b))**	459415-01

Study Title	Summary	MRID No.
Assessment of the Environmental Fate of the Cry3Bb1 Protein in Corn Fields Planted with MON 863 (Interim Report)	This interim report summarizes study progress through 2003 and includes information concerning site selection, soil characterization assays, soil specimen collection, and agronomic activities that occurred in 2003. Analysis of soil specimens for the presence of Cry3Bb1 protein has not yet been performed. **Classification: Acceptable** **(Reviewed in U.S. EPA (2006a))**	462001-01
Evaluation of Dietary Effects of Cry3Bb1 Protein on the Ground Beetle, *Poecilus chalcites* (Coleoptera: Carabidae)	First instar larvae of the ground beetle (*Poecilus chalcites*) were fed artificial diet containing Cry3Bb1 protein (930 µg/g), a 10 millimolar (mM) sodium carbonate/bicarbonate buffer control (0.147 milliliters per gram (mL/g)), a potassium arsenate reference material (200 µg/mL), or a diet-only negative control for 28 days. There were no statistically significant differences in survival, growth, or development among larvae in the Cry3Bb1, buffer control, or negative control groups. **Classification: Acceptable** **(Reviewed in U.S. EPA (2010c))**	464799-04
Evaluation of Dietary Effects of a Cry3Bb1 Protein Variant on Minute Pirate Bugs (*Orius insidiosus*)	*Orius insidiosus* nymphs were exposed for 14 days to a Cry3Bb1 protein variant (930 µg active ingredient/g) diet, a potassium arsenate (8.9 µg active ingredient/g) positive control diet, an E64 protease inhibitor (53 µg/g) positive control diet, a sodium carbonate/bicarbonate buffer control diet, or a negative control diet. There was no statistically significant difference in survival among the Cry3Bb1, negative control, or buffer control diet groups, while survival in the positive control diet groups was 0%. The percent of nymphs developing to adults and the mean number of days to develop to adults were comparable among the Cry3Bb1, negative control, and buffer control diet groups. **Classification: Acceptable** **(Reviewed in U.S. EPA (2010c))**	464799-05
Research on the Effects of Corn Rootworm Protected Transgenic Corn on Nontarget Organisms: Publications and Manuscripts	Several studies were conducted between 2000 and 2002 to evaluate the effects of the transgenic corn MON 863, which contains Cry3Bb1 protein, on non-target organisms. These studies included broad field surveys of surface-dwelling and soil-dwelling arthropods and other invertebrates, field studies of the abundance and community structure of Collembola and carabids, and laboratory and field studies of soil mites. In general, no consistent adverse effects on non-target taxa were found, although most of the studies used small test plot sizes. **Classification: Supplemental** **(Reviewed in U.S. EPA (2010c))**	462627-02

Study Title	Summary	MRID No.
Statistical Power Analysis of a Two-Year Field Study Evaluating the Ecological Effect of Corn Event MON 863	The statistical power of a previous two-year field study (MRID No. 457916-01) to evaluate the effects of MON 863 corn on terrestrial invertebrate populations was determined. For the analysis, a biologically significant effect was defined as a 50% difference in the mean abundance of individual invertebrate taxa between MON 863 and control corn plots, and a statistical power of ≥80% to detect the biological effect was desired. Single-year comparisons did not achieve the desired power level of ≥80% for detecting a 50% difference in population size for 38 of 68 (56%) comparisons made. An analysis of joint data for both years, however, found ≥80% power was obtained for 30 of 32 comparisons, and the remaining two reached 74% and 77%. The findings indicate that the experimental design used in the two-year field study was adequate to detect a 50% difference in abundance of invertebrates between plots of MON 863 and conventional corn. **Classification: Supplemental** **(Reviewed in U.S. EPA (2010c))**	462627-03
Supplemental Statistical Analysis of Data from a Two-Year Field Census Study with Corn Event MON 863	The statistical power of a previously conducted two-year field study to evaluate the effects of MON 863 corn on terrestrial invertebrate populations was determined. For the analysis, a biologically significant effect was defined as a 50% difference in the mean abundance of individual invertebrate taxa between MON 863 and control corn plots, and a statistical power of ≥80% to detect that biologically significant effect was desired. A previous statistical power analysis (MRID No. 462627-03) showed that analyzing data for each of the two years separately failed to achieve the 80% level of power in more than half of the statistical comparisons between MON 863 and RX 670 plots. The present study repeated the statistical analysis with the data for both years pooled and analyzed jointly. Results showed that the joint analysis achieved a ≥80% power level for 30 of 32 group comparisons, sufficient to determine that the original field study design was adequate for its intended purpose. **Classification: Supplemental** **(Reviewed in U.S. EPA (2010c))**	463942-02

Study Title	Summary	MRID No.
Environmental Fate of Cry3Bb1 Protein in Corn Fields Planted with MON 863	Soil samples were collected from six field sites, representing seven different soil types, in six different U.S. Corn-Belt states. Prior to study initiation, none of the plots had ever been planted in MON 863. Sampling occurred at planting, 30, 60, and 90 days after planting, six weeks after harvest, and prior to the following year's planting. Field treatments were the following: MON 863 corn with tillage; no-till MON 863 corn; RX670 corn with tillage (negative control); or no-till RX670 corn (negative control). Soil samples that were collected from the treated plots before, during, and after corn production were analyzed for persistence and accumulation of Cry3Bb1 protein using ELISA (LOQ 0.1 µg/g soil) and a Colorado potato beetle (CPB) mortality bioassay (limit of detection (LOD) 20 µg Cry3Bb1/g soil). ELISA did not detect Cry3Bb1 in any soil sample, and the CPB bioassay showed now statistically significant differences, between MON 863 and RX670 negative plots, that were attributable to the presence of Cry3Bb1. These results suggest that Cry3Bb1 protein did not persist or accumulate in soil to levels that could be detected by ELISA and/or affect the mortality of the Colorado potato beetle. If the field-based, three-year soil degradation study that is to be submitted in support of the MON 810 x MON 863 stack suggests that there is persistence and/or accumulation of Cry3Bb1 protein in soil samples, the MON 863 field-based soil degradation study should be repeated using soil samples collected from fields on which MON 863 has been grown for three consecutive years. **Classification: Acceptable** **(Reviewed in U.S. EPA (2006b))**	465103-01

Study Title	Summary	MRID No.
Field Studies Assessing Arthropod Nontarget Effects in *Bt* Transgenic Crops	This submission includes complete references and additional information that has become available on field data regarding possible impacts of *Bt* corn on non-target invertebrates. Most papers focused on large-scale field studies using transgenic corn, including discussions of study design and non-target invertebrate populations. EPA has reviewed the submitted information and a discussion summary of each paper, relevant to MON 863, is provided below. Overall, the published literature did not report any consistent adverse impacts on non-target invertebrates as a result of multi-year commercial *Bt* corn cultivation. Slight reductions in some invertebrate predator populations are seen; however, these are an inevitable result of all pest management practices, which tend to result in reductions in the abundance of the pests as prey. The continually expanding body of literature provides EPA, academia, and the public with a better understanding of the impact of transgenic crops on non-target organisms and provides useful information and considerations for those conducting large-scale field studies. (1) Bhatti *et al*. (2005a) compared ground-dwelling invertebrate abundance between Bt (MON863, Cry3Bb1) and non-*Bt* corn. Invertebrates were collected over a three year period using pan and pitfall traps. Of the 14-taxa collected, only two resulted in significant *Bt* treatment effects. However, the observed effects were not consistent and varied within and among years. (2) In a companion study to Bhatti *et al*. (2005a), Bhatti *et al*. (2005b) looked at the abundance of foliage-dwelling arthropods in *Bt* (MON863, Cry3Bb1) corn and non-*Bt* corn. Arthropods were collected over a three-year period using sticky traps. No consistent adverse impacts of *Bt* corn on the relative abundance of seven orders and 17 families of arthropods were observed. (3) Bitzer *et al*. (2005) was conducted over two years and evaluated the diversity and abundance of surface-active and subsurface springtails (Collembola) in Iowa and Illinois. Springtails were collected using pitfall traps and coil cores in plots planted with *Bt* (Cry3Bb1) and non-*Bt* corn. Using several measurements of diversity, the authors found no significant differences in the abundance of individual species between *Bt* and non-*Bt* plots. **(Reviewed in U.S. EPA (2010d))**	467129-01

3. MON 863 x MON 810 (OECD Unique Identifier: MON-ØØ863-5 x MON- ØØ81Ø-6) Expressing Cry3Bb1 and Cry1Ab

a. Data Cited/Submitted for Initial Registration of MON 863 x MON 810 (Prior to October 2003) (U.S. EPA 2003h)

MON 863 x MON 810 was produced by conventional breeding of single PIP trait corn lines MON 810 (YieldGard® Corn Borer) and MON 863 (YieldGard® Rootworm).

Non-target beneficial insect data were waived because the susceptibility of target pests to MON 863 x MON 810 is comparable to their susceptibility to the single trait Cry1Ab and Cry3Bb1 corn. Therefore, the non-target data and the environmental risk assessment for the single PIP trait corn lines are applicable to the MON 863 x MON 810 corn line (Cry3Bb1 – see section II(C)(2) of this BRAD; Cry1Ab – see U.S. EPA (2001b and 2010e)). Since there is no change in susceptibility among susceptible insects, then it is unlikely that there will be a difference in effects of the stacked versus single-trait hybrids on non-target insects.

i. Protein Interaction (MRID No. 460697-01)

Studies were conducted and submitted to test the hypothesis that the Cry1Ab and Cry3Bb1 proteins do not interact when combined in MON 863 x MON 810. It was concluded from leaf disk, whole plant, and *in vitro* studies with purified *Bt* protein that there are no interactive effects on susceptible insect pests when the Cry1Ab and Cry3Bb1 proteins are combined in MON 863 x MON 810. Since combining these proteins in MON 863 x MON 810 does not change the level of susceptibility of susceptible pests compared to the single-traits (MON 810 and MON 863 corn), it can be concluded that there will not be a difference for non-target insects not susceptible to the Cry1Ab or Cry3Bb1 proteins. As was required for the single PIP trait products, however, EPA is requiring non-target invertebrate field studies and Cry protein field degradation studies on MON 863 x MON 810.

*Note for 2010: There is an update to this summary. See section II(C)(3)(b) ("Terms and Conditions of the MON 863 x MON 810 Registration (October 2003 – September 2010)") of this BRAD.

b. Terms and Conditions of the MON 863 x MON 810 Registration (October 2003 – September 2010)

When MON 863 x MON 810 was first registered on October 31, 2003, the Agency issued a registration notice to Monsanto that, given the conclusions set forth in the initial environmental risk assessment (see section II(C)(3)(a) of this BRAD), contained the following two requirements for further environmental effects data:

> "Submit small and large-scale field studies…conducted with YieldGard® Plus Corn with appropriate end points and statistical power to verify there are no adverse ecological effects to non-target invertebrate populations…."

> "Submit field degradation studies evaluating accumulation and persistence of Cry1Ab and Cry3Bb1 from YieldGard® Plus Corn in several different soils in various strata. Representative fields must have been planted with YieldGard® Plus Corn for at least three consecutive years and include both conventional tillage and no-till samples and be harvested under typical agronomic conditions. Sampling must begin after three consecutive years of YieldGard® Plus Corn planting and continue until the limit of detection is reached. Studies should include soils with high levels of a variety of clays. Both ELISA and insect bioassays need to be conducted and compared to determine if Cry1Ab and Cry3Bb1 are accumulating or persisting in soil samples…a final report is due November 15, 2007."

For the MON 863 x MON 810 registration, the abovementioned requirements for additional environmental effects data have been satisfied by submission of appropriate studies; summaries of the studies are presented in Table 7.

Table 7. Environmental Effects Data Submitted in Response to Conditions of Registration for MON 863 x MON 810 (Reviewed in U.S. EPA (2009)).

Study Title	Summary	MRID No.
Two-Year Field Assessment of the Effect of Combined Trait *Bt* Corn MON 863 x MON 810 on Nontarget Organisms	Field trials were conducted in 2001 and 2002 at different sites each year in Warren County near Monmouth, Illinois. A split-plot design was used with four block replications. The main plots were MON 863 x MON 810, MON 863, MON 810, and RX670, and the subplots contained four insect regimes: no insecticide, seed insecticide applied before planting, soil insecticide applied at planting, and foliar insecticide. Data were collected using pan traps (for sampling soil-dwelling invertebrates), pitfall traps (for sampling surface-dwelling invertebrates), and sticky traps (for sampling foliage-dwelling invertebrates). In addition, earthworm sampling was conducted by digging soil cores and sorting through soil samples; there were no significant effects of corn lines or insecticide regimes on the abundance of earthworms in both 2001 and 2002. The results of the two-year field study consistently showed that the combined-trait *Bt* corn MON 863 x MON 810 did not have any significant adverse effects on the abundance of diverse groups of non-target taxa including predators, parasitoids, and decomposers or detritivores in comparison to non-*Bt* corn (RX670), as well as the single-trait *Bt* corn controls (MON 863 and MON 810). In contrast, insecticides applied under varying regimes had significant impacts not only on target and non-target corn pests, but also on diverse groups of predators, parasitoids, and decomposers. Although statistically significant interactions between corn lines, insecticide regimes, and/or dates were detected for a few non-target taxa, these interactions were not consistent between years and are not considered biologically relevant. The combined-trait *Bt* corn (MON 863 x MON 810), as well as the single-trait *Bt* corn lines, consistently exhibited little impact on the abundance and diversity of beneficial insects captured during the two-year field study. **Classification: Acceptable**	472829-01
Environmental Fate of the Cry3Bb1 and Cry1Ab Proteins in Corn Fields Planted with MON 863 x MON 810 for Three Consecutive Years	Field studies at six sites (AR, CO, IA, IL, MN, and NE) with a range of soil properties and two sampling depths (0–6 and 6–12 inches) had no detectable Cry3Bb1 or Cry1Ab protein in the soil after 3 years of continuous cropping with YieldGard Plus® Corn (Mon 863 x MON 810) as determined by ELISA analysis or soil dietary bioassay with CPB or European corn borer (ECB) neonates. This 3-year field study is consistent with earlier studies indicating no accumulation or persistence of the Cry3Bb1 and Cry1Ab proteins in soils. There is negligible potential for plant-produced Cry3Bb1 and Cry1Ab proteins to persist or accumulate in agricultural soils across cropping cycles with YieldGard Plus® Corn. **Classification: Acceptable**	472829-02

4. MON 88017 (OECD Unique Identifier: MON-88Ø17-3) Expressing Cry3Bb1 and MON 88017 x MON 810 (OECD Unique Identifier: MON-88Ø17-3 x MON- ØØ81Ø-6) Expressing Cry3Bb1 and Cry1Ab

a. Data Cited/Submitted for Initial Registrations of MON 88017 and MON 88017 x MON 810 (Prior to December 2005)(U.S. EPA 2005a and 2005b)

i. Background

The Cry3Bb1 protein expressed in MON 88017 is a variant of the wild-type Cry3Bb1 protein from *Bt* subsp. *kumamotoensis* that protects the roots of corn plants from feeding damage caused by the coleopteran pest, corn rootworm (*Diabrotica* spp.). Previously described product characterization studies (see section II(A)(4) of this BRAD) demonstrated the functional and physicochemical equivalence of the Cry3Bb1 protein expressed in MON 88017 with the Cry3Bb1 protein expressed in Corn Event MON 863. That is, the Cry3Bb1 proteins expressed in both Corn Event MON 863 and MON 88017 share an amino acid sequence identity of >99.8%, differ from one another by only one of 653 amino acids, express similar protein levels in plant tissues, show similar biological activity against larvae of the Colorado potato beetle and western corn rootworm, and exhibit comparable field efficacy against corn rootworm. Given the similarities between the two proteins, all environmental effects data submitted to support Corn Event MON 863 were bridged to MON 88017.

ii. MON 88017 (Coleopteran-Active Cry3Bb1 Protein) Risk Assessment

For registration of MON 863 (also applicable to MON 88017), EPA reviewed studies conducted on representative non-target species with several Cry3Bb1 protein variants and performed risk assessments on plants, wild mammals, birds, fish, aquatic invertebrates, estuarine and marine animals, earthworms, terrestrial non-target insects (including honey bee adults and larvae, parasitic wasps, green lacewings, several lady beetle species, springtails (collembola toxicity/reproduction), and monarch butterflies), field evaluations of the effects of Cry3Bb1 exposure on non-target invertebrates, soil degradation/persistence, and endangered species (see section II(C)(2) of this BRAD). In addition, gene flow and weediness assessments, via pollen and Cry protein DNA uptake by plants and soil microorganisms, were also performed. The Agency has sufficient information to believe that there is no risk from the proposed uses of Cry3Bb1 corn to non-target wildlife, aquatic, and soil organisms. The Agency has requested additional data in hopes that it will provide weight to the some of the initial conclusions made regarding use of Cry3Bb1 corn. These additional data consist of long-range soil fate studies, long-range field effects on invertebrates studies, and toxicity studies on additional Coleoptera, specifically the ground beetle and the minute pirate bug. Whether any additional non-target or long-range field studies are required on MON 88017 will be determined by the results of these reviews. In the event that these studies sufficiently demonstrate a lack of long-range adverse effects, no additional data will be required. The evaluation of the submitted Cry3Bb1 long-range field studies will be based on recommendations from the August 27, 2002 FIFRA SAP.

*Note for 2010: There is an update to this summary. See section II(C)(4)(b) ("Terms and Conditions of the MON 88017 and MON 88017 x MON 810 Registrations (December 2005 – September 2010)") of this BRAD.

iii. MON 810 (Lepidopteran-Active Cry1Ab Protein) Risk Assessment

Likewise, the EPA has conducted an extensive review of effects of the Cry1Ab protein present in MON 810 to non-target organisms in an ecological risk assessment as part of the reassessment of *Bt* plant-incorporated-protectants (U.S. EPA 2001b). EPA reviewed studies conducted on representative non-target species with Cry1Ab protein and performed risk assessments on plants, wild mammals, birds, fish, aquatic invertebrates, estuarine and marine animals, earthworms, terrestrial non-target insects (including honey bee adults and larvae, parasitic wasps, green lacewings, lady beetles, springtails (collembola toxicity/reproduction), monarch butterflies, and black swallow tail butterflies), field invertebrate abundance, soil degradation/persistence, and endangered species with special emphasis on the Karner blue butterfly. In addition, weediness and gene flow assessments, via pollen and Cry protein DNA uptake by plants and soil microorganisms, were also performed. The Agency concluded, considering all available information, the weight-of-evidence indicates no unreasonable adverse effects of *Bt* Cry proteins in plants to non-target wildlife, plants, or beneficial invertebrates (U.S. EPA 2001b).

iv. MON 88017 x MON 810 Corn (Cry3Bb1 x Cry1Ab Coleopteran/Lepidopteran-Active Protein) Risk Assessment

The YieldGard® Plus Corn (Cry protein content equivalent to MON 88017 x MON810 corn) did not enhance or diminish European corn borer (ECB), corn earworm (CEW), fall armyworm (FAW), or southwestern corn borer (SWCB) leaf feeding damage compared to single-trait MON 810 corn containing the Cry1Ab protein in five *in planta* assays. YieldGard® Plus Corn also did not enhance or diminish western corn rootworm (WCRW) and southern corn rootworm (SCRW) larval feeding on roots compared to single-trait MON 863 corn containing the Cry3Bb1 protein. Leaf disk assays resulted in no difference in insecticidal activity against FAW between YieldGard® Plus Corn and single-trait MON 810 corn. The presence of Cry3Bb1 in YieldGard® Plus Corn did not affect FAW nor did the presence of Cry1Ab affect Colorado potato beetle (CPB) in leaf disk assays. Insect bioassays conducted with purified protein verified that Cry3Bb1 will not affect ECB survival, and Cry1Ab will not affect CPB survival. LC_{50} values for ECB and CPB were similar for the single-trait hybrids (MON 810 and MON 863) and dual-trait hybrid (Cry3Bb1 x Cry1Ab), and dose response curves did not differ.

Collectively, these data provide evidence that the Cry1Ab and Cry3Bb1 proteins do not interact in an antagonistic, additive, or synergistic manner. Results of these assays verify that no interactive affects occur (which was expected), since different physiological conditions are needed for the Cry1Ab and Cry3Bb1 proteins to function. Protection against lepidopteran and coleopteran target pests were equivalent for the single-trait and stacked hybrids. Based on the lack of interactive effects on susceptible pests, it is extremely unlikely that the Cry3Bb1 and Cry1Ab proteins contained in a single plant will impart any safety concerns for non-target organisms exposed to these hybrids in the environment.

It can be concluded from the leaf disk, whole plant, and *in vitro* studies with purified *Bt* protein that there are no interactive effects on susceptible insect pests when the Cry1Ab and Cry3Bb1 proteins are combined in YieldGard® Plus Corn. Since combining these proteins in YieldGard® Plus Corn does not change the level of susceptibility of susceptible pests compared to single-trait MON 810 and MON 863 corn, it can be concluded that there will not be a difference for non-target insects not susceptible to the Cry3Bb1 or Cry1Ab proteins. Therefore, development of a new set of non-target organism effects data were waived for MON 88017 x MON 810 corn.

The Agency has sufficient information to believe that there is no risk from the proposed uses of MON 88017 x MON 810 corn to non-target wildlife, aquatic, and soil organisms. The Agency has requested additional data on Cry3Bb1 in corn (Corn Event MON 863); these supplementary studies should provide weight to the Agency's initial conclusions regarding the use of Cry3Bb1 corn. The additional data consist of long-range soil fate studies, long-range field effects on invertebrates studies, and toxicity studies on additional Coleoptera, specifically the ground beetle and the minute pirate bug. In the event that these studies sufficiently demonstrate a lack of long-range adverse effects, no additional data with MON 88017 x MON 810 will be required. Cry1Ab protein levels in MON 810 and MON 88017 x MON 810 young root and forage root are required regardless of the outcome of the MON 863 studies being conducted to fulfill conditions of registration.

*Note for 2010: There is an update to this summary. See section II(C)(4)(b) ("Terms and Conditions of the MON 88017 and MON 88017 x MON 810 Registrations (December 2005 – September 2010)") of this BRAD.

b. Terms and Conditions of the MON 88017 and MON 88017 x MON 810 Registrations (December 2005 – September 2010)

When MON 88017 and MON 88017 x MON 810 were initially registered on December 13, 2005, the Agency issued registration notices to Monsanto that contained the following requirements in relation to further environmental effects data:

- For MON 88017 –

  "Submit all data required to support the individual plant-incorporated protectant in Event MON 863 (YieldGard Rootworm), 524-528. In the event that the Agency concludes MON 863 (YieldGard Rootworm) studies do not sufficiently demonstrate a lack of significant adverse effects, additional data with MON 88017 corn must be submitted. These data may include a) laboratory toxicity testing with *Orius insidiosus* (minute pirate bug), b) laboratory toxicity testing with a carabid (ground beetle), c) long range effects testing on invertebrate populations in the field, and d) long range soil persistence testing."

- For MON 88017 x MON 810 –

  "Submit all data required to support the individual plant-incorporated protectant in MON 810 (YieldGard), Event MON 863 (YieldGard Rootworm), MON 88017 corn EPA Registration Nos. 524-489, 524-528. In the event that the Agency concludes MON 863 (YieldGard Rootworm) studies do not sufficiently demonstrate a lack of significant adverse effects, additional data with MON 88017 x MON 810 corn must be submitted. These data may include a) laboratory toxicity testing with *Orius insidiosus* (minute pirate bug), b) laboratory toxicity testing with a carabid (ground beetle), c) long range effects testing on invertebrate populations in the field, and d) long range soil persistence testing."

  "Submit expression level data regarding Cry1Ab protein levels in MON 810 and MON 88017 x MON 810 young root and forage root within 12 months of the date of registration."

All requirements for additional environmental effects data for MON 810 (see U.S. EPA (2010e)) and Corn Event MON 863 (see section II(C)(2)(b) of this BRAD), as set forth in the December 13, 2005 registration notices, have been satisfied for both registrations (see Table 8). Moreover, after evaluating the conditional data submitted to support the registration of Corn Event MON 863, the Agency has concluded that these data do not demonstrate the potential for long-range adverse effects to the environment as a result of the cultivation of Cry3Bb1 corn.

Table 8. Environmental Effects Data Submitted in Response to Conditions of Registration for MON 88017 x MON 810 (Reviewed in U.S. EPA (2010a)).

Study Title	Summary	MRID No.
Assessment of Cry1Ab Protein Levels in Corn MON 88017 x MON 810 Root Tissue Produced in U.S. Field Trials in 2006	A traditionally crossed corn hybrid of MON 88017 with MON 810 was grown along with conventional seed and MON 810 corn at five locations in 2006 using a randomized complete block design and sampling scheme. Young root tissues were sampled at V2–V3 and forage root tissues at early dent or 1/3 milkline. Samples were stored and shipped on dry ice for Cry1Ab analysis of trypsinized, extracted tissues. Extraction efficiency was 92%, spike recovery was 77%, and the trypsinization factor was 2. The coefficient of variation was 14% between assays. Limit of detection was 0.13 µg/g fresh weight, and limit for quantification was 0.40 µg/g fresh weight. ELISA revealed mean Cry1Ab protein levels in MON 88017 x MON 810 corn tissues across all sites were 75 µg/g dry tissue weight (dwt) in young root and 12 µg/g dwt in forage root; similar to the mean Cry1Ab protein levels in MON 810 corn, which were 78 µg/g dwt in young root and 13 µg/g dwt in forage root. **Classification: Acceptable**	470045-01

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D. Insect Resistance Management (IRM)

1. Background

Corn expressing the Cry3Bb1 protein provides protection against certain species of the corn rootworm (CRW), including western corn rootworm (WCRW, *Diabrotica virgifera virgifera*), northern corn rootworm (NCRW, *Diabrotica barberi*), and Mexican corn rootworm (MCRW, *Diabrotica virgifera zea*). In order to delay the onset of insect resistance to Cry3Bb1 corn, an acceptable IRM plan is necessary. The IRM plan consists of the following: (1) a 20% structured refuge placed adjacent to or within the YieldGard® Rootworm corn (MON 863) field, in-field strips must be at least four rows wide; (2) a resistance monitoring program; (3) a remedial action plan; and (4) an IRM grower compliance and education program. The Environmental Protection Agency (EPA) also required that Monsanto Company ("Monsanto") provide to the Agency annual resistance monitoring, compliance (and education), and sales reports.

2. MON 863 (YieldGard® Rootworm; EPA Reg. No. 524-528)

a. Regulatory Background

MON 863 was registered for commercial use for the 2003 growing season and is targeted against corn rootworm (CRW, *Diabrotica* spp.). Prior to registration, Monsanto submitted several documents in support of an interim Cry3Bb1 IRM plan. An IRM plan for MON 863 corn dated June 20, 2000 was submitted to the Agency (Master Record Identification Number (MRID No.) 451568-05). This submission included information on dose, CRW biology, simulation models of resistance development, and grower surveys. Research reports and results of grower surveys were also included in the appendices of the June 2000 submission. An amended IRM plan dated January 8, 2002 was submitted to the Agency for review (MRID No. 455770-01). The amended plan titled "An Interim Insect Resistance Management Plan for Corn Event MON 863: A Transgenic Corn Rootworm Control Product" was intended to supersede MRID No. 451568-05. Therefore, MRID No. 451568-05 was used for additional information and as reference material but was not formally reviewed (see review of interim IRM plan in U.S. EPA (2002a)). An additional preliminary research report dated February 20, 2001 was submitted to the Agency by Monsanto (MRID No. 453484-01).

A Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) was convened in August 2002. The August 2002 SAP comments regarding Monsanto's interim IRM plan were documented in a memorandum from Paul Lewis to Marcia Mulkey dated November 6, 2002 (U.S. EPA 2002b). In response to the SAP, Monsanto submitted additional information to EPA in a document from Dennis Ward to Janet Andersen dated December 13, 2002 (Ward 2002). This additional information, along with additional clarifications provided to the Agency by Dr. Michael Caprio on December 20, 2002, Dr. David Andow on December 23, 2002, and Dr. Fred Gould on February 12, 2003, was incorporated into the final review (U.S. EPA 2003a).

As terms and conditions of registration in 2003, Monsanto was required to submit resistance allele frequency data and additional IRM research data to increase the understanding of corn rootworm biology and other factors related to resistance management. The following areas of research were to be addressed:

- Research regarding adult and larval movement and dispersal, mating habits, ovipositional patterns, number of times a female can mate, and fecundity;
- Research to determine if IRM strategies designed for WCRW and NCRW are appropriate for MCRW;
- Research regarding the mechanism of potential resistance of CRW to MON 863 is necessary to develop an appropriate long-term IRM strategy. Monsanto must attempt to develop resistant CRW colonies to aid in determining selection intensity;
- Research regarding the effect of WCRW ovipositing in soybean prior to overwintering and extended diapause in NCRW on an IRM strategy needs further investigation;
- Detailed summaries of the four data-sets identified in Monsanto's December 13, 2002 letter should be submitted to the Agency to support their conclusion that the initial resistance allele frequency is ≤ 0.01;
- Baseline susceptibility studies currently underway should be continued for WCRW and initiated for NCRW and monitoring techniques, such as discriminating dose concentration assays, need to be thoroughly investigated for their feasibility as resistance monitoring tools.

Monsanto was to provide protocols for the proposed research (within 90 days of the date of registration) and a progress report by January 31, 2004. A final report was to be submitted by January 31, 2006.

Monsanto submitted 12 protocols for review (submitted May 23, 2003; no MRID No.) and a progress report (submitted January 30, 2004; MRID No. 461865-01) covering the first four research areas; both the protocols and the 2004 progress report were found to be acceptable (U.S. EPA 2004c).

In April 2005, Monsanto submitted a second progress report, providing the Agency with an update of the ongoing insect resistance management research. This second progress report (MRID No. 466066-01) was found to be acceptable (U.S. EPA 2006b). The final report (summarized below) was submitted January 24, 2006 and was reviewed in U.S. EPA (2006c).

Monsanto submitted a full report on four data sets regarding the initial Cry3Bb1 resistance allele frequency in CRW. Although this report satisfied the condition of registration, there is still significant uncertainty regarding the mode of action of Cry3Bb1 corn, the nature of potential CRW resistance, and the frequency of resistance allele(s) in pest populations (U.S. EPA 2004d).

Summary of Final IRM Research Submissions

Monsanto submitted fourteen studies to provide additional information on the biology and ecology of the corn rootworm pest complex. They are divided into the following subject areas: CRW larval movement (Hibbard *et al*. 2003; Hibbard *et al*. 2004; Hibbard *et al*. 2005), CRW adult movement (Spencer *et al*. 2003; Kim and Sappington 2005), CRW hosts (Clark and Hibbard 2004; Oyediran *et al*. 2004; Wilson and Hibbard 2004), rotation-resistant WCRW (Rondon and Gray 2004; Crowder *et al*. 2005; Onstad *et al*. 2003), extended diapause NCRW (Mitchell and Onstad 2005), and baseline susceptibility of CRW to Cry3Bb1 (Siegfried *et al*. 2005). Data from the CRW larval movement studies have been used to make changes to the in-field refuge strip width requirement for YieldGard® Rootworm, YieldGard® Plus, MON 88017, and MON 88017 X MON 810 (i.e., in-field strip width was changed from at least 6 to 12 rows wide to at least 4 rows wide). A list of the literature citations for the studies is provided in the "References" section of this IRM chapter. Findings from these studies are incorporated into the next section of this document, "Corn Rootworm Biology and Factors Related to Resistance Management."

b. Corn Rootworm Biology and Factors Related to Resistance Management

i. Pest Biology (Information Considered in the Original Risk Assessment)

This section contains data and information reviewed as part of the original IRM risk assessment for MON 863 corn. Pest biology data that were required as a condition of registration are discussed in the subsequent section of this document.

In order to develop an appropriate IRM strategy for MON 863 corn, as well as all insect-protected transgenic crops, it is important to consider the biology of the target pest. Knowledge of pest biology is imperative in determining optimal size and placement of refuges that will encourage random mating between pests in *Bt* and non-*Bt* corn fields. Based on the movement of CRW adults, a non-*Bt* corn refuge should be planted adjacent to or within MON 863 fields.

Characteristics of pest biology that are relevant to IRM (e.g., movement, feeding habits, and ovipositional habits) differ for WCRW and NCRW. WCRW and NCRW adults will feed on corn silks, pollen, and young kernels in the ear tip; however, only WCRW feed on leaves. Since NCRW adults do not feed on corn leaves, they leave the field after pollination to find a field with pollen available (Branson and Krysan 1981). Since adult and larval CRW feed on various parts of the corn plant, both life stages may be exposed to the *Bt* protein and extended selection pressure may result (Meinke *et al*. 2001). Severe root damage from larval feeding will lead to plant lodging (where damaged corn stocks fall over making mechanical harvesting impossible) and yield losses.

WCRW and NCRW are univoltine in most of the Corn Belt (Branson and Krysan 1981; Meinke *et al*. 2001). CRW typically oviposit where the adults are feeding, which is almost exclusively in corn fields (Branson and Krysan 1981; Levine and Oloumi-Sadeghi 1991). In general, CRW adult emergence varies based on species, geography, weather, management practices (such as insecticide use), population

density, and sex. For instance, males typically emerge before females, and emergence, as well as fecundity, longevity, and egg viability, are reduced in corn planted later in the season (Boetel and Fuller 1997; Levine and Oloumi-Sadeghi 1991; Meinke *et al*. 2001). It is unknown what effect corn rootworm-protected transgenic corn will have on phenology, sex ratio, and adult emergence patterns. Asynchronous adult emergence for *Bt* corn fields and non-*Bt* refuges may lead to nonrandom or assortative mating, which may lead to an increase in the rate of resistance evolution. Nonrandom or assortative mating may also occur if *Bt* corn disrupts the synchrony of male and female CRW adult emergence (Meinke *et al*. 2001). Mating typically occurs within 24 to 48 hours of female adult emergence within the corn fields they emerged from or nearby (Meinke *et al*. 2001).

CRW larval movement is limited, particularly in areas with low population densities (Meinke *et al*. 2001). Published and unpublished articles have reported varying distances that CRW larvae move. WCRW larvae may move from 12 to 16 inches and have been found in corn rows planted up to 40 inches apart (Suttle *et al*. 1967; Short and Luedtke 1970; Gray 1999). These studies suggest that CRW larvae hatching from eggs between rows are capable of finding and injuring corn roots regardless of row spacing. Since field corn is typically planted approximately 24 to 30 inches apart, CRW may move up to two rows according to current research. Additional information is needed, however, to verify the distance CRW larvae move within and between rows. In general, young CRW larvae (e.g., 1st, 2nd, and sometimes 3rd instars) tend to move toward actively growing corn roots. Larval movement toward respiring, growing corn roots is probably because of their ability to detect and move toward carbon dioxide sources (Strnad and Bergman 1987; Gray 1999). Young larvae will feed on the distal portion of corn roots and move through the soil to feed on new, short roots as they develop into later instars (Strnad and Bergman 1987; Gray 1999). It is possible, therefore, that a RS heterozygous larva with a partially recessive resistance trait will begin feeding on transgenic corn roots and finish its development on adjacent non-transgenic roots, which would result in a non-lethal dose of MON 863 and potential survival of that larva.

NCRW- and WCRW-mated adults may be very mobile and have potentially high dispersal capabilities (Meinke *et al*. 2001). Nonetheless, local dispersal is more common and involves movement within or among adjacent fields; migratory dispersal over long distances occurs in a small portion of individuals and usually involves females (Meinke *et al*. 2001). Dispersal capabilities of the WCRW are greater than the NCRW. The WCRW is also a greater competitor and displaced the NCRW in Nebraska by 1980 (Hill and Mayo 1980). WCRW post-mating dispersal may be local or migratory. Published data suggest that some WCRW females may leave the field after mating to oviposit elsewhere (Coates *et al*. 1986). While sustained flights by mated female CRW are possible, movement by unmated females is limited. Knowledge of the maximum and average distance an adult CRW moves is limited. Additional research, regarding adult and larval WCRW and NCRW dispersal potential, is needed to determine placement of non-*Bt* corn refuges.

Additional information was required on various aspects of CRW pest biology as it relates to a long-term IRM strategy. Characteristics of pest biology that are relevant to IRM (e.g., movement, feeding habits, and ovipositional habits) differ for WCRW and NCRW; therefore, additional information on the biology

of the WCRW and NCRW was needed. According to the August 2002 SAP, the WCRW and MCRW are subspecies and much of the data collected on biology will relate to both species. The Panel concluded, however, that data on adult mating behavior, male and female migration, and reproductive biology and fecundity of females would be needed to determine if the IRM plan is suitable for MCRW. Although the SAP concluded that the same IRM strategy may be appropriate for the WCRW and NCRW, the Panel recommended additional research on the NCRW and suggested collecting data from several geographic locations of the WCRW. There are behavioral differences in WCRW populations from the western and eastern regions of their distribution. Thus, studies on aspects of pest biology (e.g., movement) should be conducted in several areas. Since the biology of the southern corn rootworm (SCRW, *Diabrotica undecimpunctata howardi*) is very different from the other *Diabrotica* spp. and it is not adequately controlled by MON 863, SCRW should not be considered.

The August 2002 SAP identified several areas of additional research needed to fully understand CRW biology as it relates to an IRM strategy. The SAP concluded that male and female adult movement, as well as fitness in MON 863 and non-transgenic corn, should be evaluated in large-scale field studies. Data needed on movement include, but are not limited to, the distance males and females will move over time and the rate adults leave the natal field. Research may also be needed on the movement of NCRW male and female adults since little is currently known. The SAP also recommended an evaluation of "the impact of adult density on migration patterns of adults, whether a delay in male emergence from MON 863 affects male fitness and lowers the chances of mating, and whether there are sublethal effects of MON 863 on female fecundity, offspring quality and other fitness parameters."

The NCR-46 (a technical committee consisting of research and extension CRW specialists and other cooperators) submitted a letter dated May 29, 2001 to the EPA that outlines additional CRW biology research. The August 2002 SAP recommended that the EPA consider the recommendations made by the NCR-46.

Based on reviews of available data and the SAP and NCR-46 recommendations, the Agency concluded that more information on CRW movement, host utilization, mating habits, ovipositional patterns, the number of times a female can mate, and fecundity would be useful for CRW IRM. In addition, further investigation of the IRM impacts of behavioral resistance adaptations, including the effects of WCRW oviposition in soybean prior to overwintering and extended diapause in NCRW, was recommended. In previous submissions (Master Record Identification Numbers (MRID Nos.) 453484-01 and 455770-01), Monsanto listed and summarized studies underway at the time of the MON 863 registration application that related to the biology of CRW. Additional CRW biology, ecology, and genetics data were required as conditions of the MON 863 (YieldGard® and YieldGard® Plus) registrations. Results of these studies, as well as additional research, were submitted to EPA after registration and are summarized in the next section.

ii. Pest Biology (Conditionally Required Studies)

As a condition of registration, Monsanto submitted fourteen studies to provide additional information on the biology and ecology of the corn rootworm pest complex. These data address the following subject areas: (1) CRW larval movement; (2) CRW adult movement; (3) CRW hosts; (4) rotation-resistant WCRW; (5) extended diapause NCRW; and (6) baseline susceptibility of CRW to Cry3Bb1 (discussed in the resistance monitoring section). Further information on some of the research objectives (i.e., applicability of the IRM plan to MCRW and development of resistant colonies) was provided in progress reports. The Agency has reviewed these data (and other published studies) and summarized the findings by discipline in this section.

CRW Larval Movement

Larval movement data published by Hibbard *et al*. (2003) show that between 0.75% and 6% of larvae moved across corn rows. This represents a relatively high-end estimate of the number of larvae that cross rows. This means that much narrower in-field strips should be sufficient to provide adequate protection from sublethal selection caused by CRW larval movement across rows and maintain low functional recessiveness. Any increase in sublethal selection would be offset by a greater probability that potentially resistant adults emerging from the *Bt* corn rows would be mated by susceptible adults from the refuge row. Single-row strips would likely be too narrow and allow too much larval movement across rows to sufficiently maintain low functional recessiveness. Therefore, in-field strips of ≥4 row strips should provide sufficient CRW resistance management within the field based both on the consideration of the current understanding of larval movement and selection, as well as grower feasibility, practicality, consistency, and compliance.

CRW larval movement is density dependent at high infestation levels (Onstad et al. 2006). According to Hibbard *et al*. (2004), however, density-dependent dispersal for neonates did not occur during a three-year field study, even under high-density pressure (with artificial infestation). Subsequent investigation by Hibbard *et al*. (2005) found that late-instar movement occurred from sufficiently damaged non-*Bt* plants to surrounding transgenic plants. Work by Dr. Hibbard indicates that it is more realistic to assume high larval movement from late instars and little to no movement for neonates. On the other hand, statistically significant neonate movement from *Bt* plants to non-*Bt* plants was observed in one year of their field study (Hibbard *et al*. 2005).

Adult CRW Movement

Spencer *et al*. (2003) developed a new technique that used ingested transgenic corn tissue as a marker for measuring movement of CRW adults between corn and soybean fields. This method used lateral flow strips to detect the Cry3Bb1 protein in the gut of insects that had ingested YieldGard® Rootworm. Insects feeding on YieldGard® Rootworm could be detected for at least 16 hours after feeding but not 32 hours. This technique allows the impact of factors, such as temperature, precipitation, and wind speed, on short-term adult movement to be studied. Spencer *et al*. (2003) found that 85.3% of males and

females moved ≤4.6–9.1 meters per day (m/d) through R2-R3 stage corn. For Cry3Bb1-positive adults that moved out of corn fields into an adjacent soybean field, 86.4% of males and 93.1% of females moved ≤4.6–9.1 m/d through soybean. Data suggest that plant-to-plant movement was motivated by a search for food and was density dependent because plant damage was density dependent. This technique is a tool to better estimate rates of beetle movement away from transgenic corn than currently employed techniques, such as fluorescent powder mark-recapture methods, and offers an opportunity to better study dispersal of CRW and other insect herbivores.

Separately, Nowatzki *et al*. (2003a and 2003b) conducted a field study (mark-recapture) with WCRW using a rubidium labeling technique to quantify short-range, in-field movement of adult beetles from the time of their emergence. The data (from trials in Nebraska) showed that males moved an average of 13.9 m/d prior to female emergence and 15.2 m/d during peak female emergence (data were pooled across two years). Females moved on average 1.9 m/d during peak female emergence and 13.1 m/d during post-female emergence (data pooled across years).

Kim and Sappington (2005) studied the population genetic structure of 10 western corn rootworm populations (595 individuals sampled) from nine U.S. states (western Texas and Kansas to New York and Delaware) based on microsatellite loci analysis. These researchers found that all populations exhibited high levels of genetic diversity, with the mean allelic diversity ranging from 7.3 to 8.6 and mean expected heterozygosity ranging from 0.600 to 0.670. Little genetic differentiation, as a whole, was observed across the geographic range sampled, with a global fixation index (F_{st}) of 0.006. Pairwise F_{st} estimates also indicated little genetic differentiation. The researchers concluded that the western corn rootworm population had not had sufficient time for substantial genetic structuring since its recent eastward range expansion from the Great Plains approximately 50 years ago.

CRW Hosts

A variety of grass species were studied to determine their suitability as a refuge host for corn rootworms. Several prairie grasses and forage grasses were shown to support larval growth of western corn rootworm larvae (Clark and Hibbard 2004; Oyediran *et al*. 2004; Wilson and Hibbard 2004) and, therefore, may serve as additional refuge to the structured non-Cry3Bb1 corn refuges. This additional source of refuge could reduce the risk of Cry3Bb1 resistance evolving.

Clark and Hibbard (2004) examined larval survivorship and growth parameters of western corn rootworm on the roots of 29 plant species comprised of maize, maize-field weeds, native prairie grasses, forage grasses, and small grain crops. Adults were recovered from five plants species in addition to maize, and larvae survived at least 6 days after infestation on 27 species and 24 days on 23 plant species.

Oyediran *et al*. (2004) evaluated 21 prairie grass species as larval hosts of western corn rootworm. Maize and sorghum were included as positive and negative controls, respectively. Overall, adults were produced from 14 of 23 species evaluated.

Wilson and Hibbard (2004) monitored larval development and survivorship of western corn rootworm on 22 plant species, including maize, maize-field weeds, and selected native prairie grasses, fence-row/forage grasses, and small grain crops planted in greenhouse trials. Adults were recovered from 10 species. Larvae survived at least 14 days on 21 species and 26 days on 18 species.

The potential for rootworm larvae to move between weeds within or adjacent to a maize field could be an important factor in resistance management of transgenic-rootworm maize. The long-term implication of such movement for a low-dose transgenic event has not yet been worked out.

Rotation-Resistance in WCRW

Traditionally, farmers rotate the planting of corn and soybeans in a field as a means to manage corn rootworm. In recent years, however, the effectiveness of this practice has been diminished because of a soybean-variant of the corn rootworm that also deposits eggs in soybean fields. Rondon and Gray (2004) studied the oviposition patterns of the soybean-variant and found that, although corn can be the preferred oviposition site among crops, similar egg densities can be found in soybean and oat stubble. These results indicate that producers who choose to rotate corn with soybean or other crops, such as alfalfa, may be at risk to economic larval injury to corn roots.

Crowder *et al*. (2005) modeled pest management strategies from both a biological and an economic perspective. Based on these modeling efforts, greater doses were the most effective at preventing resistance to transgenic corn with the standard management strategies. This was especially true in areas without rotation-resistant phenotypes. Returns with the dynamic adoption strategies were always similar when compared with the standard strategy with a medium or greater dose. If the pest management industry can achieve a high dose of toxin, farmers can plant 80% of their corn fields to a transgenic cultivar with confidence that this strategy will be beneficial biologically and economically. Results indicate that, in areas without rotation-resistance, planting 80% transgenic corn (required refuge is 20%) in the continuous corn field each year generated the greatest economic returns with a medium toxin dose or greater. Where rotation-resistance was a problem, planting transgenic corn in the rotated corn field was the most effective strategy. These results support the current IRM strategy for MON 863.

Onstad *et al*. (2003) also modeled management strategies for rotation-resistance over a 15-year timeframe and concluded that using corn rootworm-resistant corn was an economically valuable approach using a 2-year or 3-year rotation strategy.

Overall, these studies indicate that YieldGard® Rootworm corn can be a useful tool for managing rotation-resistance. Furthermore, the existing IRM plan, using a 20% refuge for YieldGard® Rootworm, should be appropriate whether or not rotation-resistant corn rootworms are present.

Extended Diapause in NCRW

Some northern corn rootworm populations have developed an extended diapause period, resulting in synchronization of egg hatch with the planting of corn and thus circumventing crop rotation management strategies. Mitchell and Onstad (2005) developed a population genetics model to study the impact of northern corn rootworm populations with extended diapause on current IRM strategies. The model produced mixed results depending on various other factors, such as insecticide use and farmer practices, but overall showed that extended diapause tended to reduce the rate of resistance evolution. No changes to the existing IRM plan for YieldGard® Rootworm corn were indicated by these modeling efforts.

Applicability of the IRM Strategy to Mexican Corn Rootworm

Although this research requirement was not addressed in the final report, information was previously submitted in the progress reports (described in MRID No. 466066-01; reviewed in U.S. EPA 2006b). Monsanto submitted the results of a MCRW field efficacy evaluation designed to (1) evaluate and compare performance of MON 863 vs. conventional soil insecticides in protecting corn roots from injury by MCRW; and (2) evaluate performance of MON 863 hybrids vs. conventional soil insecticides and experimental seed treatments in protecting corn roots from MCRW.

Of the 12 efficacy trials conducted, four were (unintentionally) planted in areas with insignificant MCRW pressure for the 2003 growing season (e.g., 0.25 Node Injury Scale (NIS) in untreated check); thus, only eight trials yielded useful data. MON 863 provided corn root damage control of MCRW greater than or equal to that provided with furrow treatment by the commercial insecticides. MON 863 provided better MCRW control than non-*Bt* isoline seed treatment with clothianidin at 1.25 milligrams (mg) active ingredient/kernel. The average NIS for MON 863 was 0.10, while MON 863 plus clothianidin was 0.07. Comparatively, the NIS for the isoline seed alone was 1.2 and the isoline plus clothianidin was 0.32. Since the summary of these results was not presented with accompanying statistical analyses, it was not possible to determine whether significant differences occurred between MON 863 alone and the highest non-*Bt* isoline seed treatment tested.

Mechanism of Potential Resistance of CRW/Development of a Cry3Bb1-Resistant Colony

EPA did not receive a final report from Monsanto detailing efforts to investigate potential resistance and develop a resistant colony. Information on this research objective, however, was provided in a previously submitted progress report (described in MRID No. 466066-01; reviewed in U.S. EPA 2006b).

The first study (conducted by P. Clark and J. Foster, Department of Entomology, University of Nebraska) was intended to identify differences in 1st, 2nd, and 3rd instar larval feeding on transgenic maize compared with its non-transgenic isoline on the basis of larval feeding behavior, neonate feeding location, and larval survivorship. The research design incorporated the novel feature of a transparent corn plant growth medium that permitted direct visual observation of WCRW larval feeding *in situ*

without disturbing the feeding larvae or growing roots. Qualitative observations (feeding location, larval movement in and around the transgenic and non-transgenic plants, etc.) were recorded, and quantitative measurements were made on root wet and dry weight, larval wet and dry weight, head capsule width, and larval mortality. Observational results showed that 1st instar larvae aggregate and initiate feeding at the root tip meristem on both transgenic and non-transgenic corn. Following this initial aggregation phase, larval behavior differs, depending on whether the plant is transgenic or not. On non-transgenic corn, larvae feed into the root interior, leaving an outer "shell" of 1–2 layers of epidermal root tissue; continued feeding results in larval movement into older and elongated root tissues over time. On MON 863 corn, 1st and 2nd instar larvae began feeding on meristematic tissue but terminated feeding before entering the root interior; further, larvae on MON 863 fed less frequently, did not become established at feeding sites, and moved more frequently than same-stage instars on conventional corn. Survival of 1st and 2nd instar larvae to successful molt on MON 863 was approximately 1%. Larvae that fed and molted to 2nd instar on transgenic roots exhibited the same growth rate as did larvae in non-transgenic isoline; further, there were no significant differences for the parameters of head capsule width and larval wet and dry weight. These results seem consistent with a "repellent factor" in roots or root exudate that may contribute to the overall efficacy of MON 863.

In the second study (conducted by T. Clark, B. Hibbard, and I. Oyediran, Department of Entomology, University of Missouri), the objective was to determine selection intensity and associated fitness parameters of WCRW on transgenic, rootworm-resistant corn in a Missouri environment. During 2004, the study was expanded to include sites in Nebraska, Iowa, and South Dakota. Replicate plots of glyphosate-resistant and MON 863 and non-MON 863 isoline corn were established at the field sites and infested at V2 with two densities of WCRW eggs. At pupation, emergence cage monitoring of each plot was performed daily and continued for 3 weeks until emergence ceased. Adults were counted and sexed, and results were analyzed for effects of environment (weather, soil information, and agronomics), infestation level, and corn type. In the experiment, selection intensity was defined as beetle emergence from a known egg load. When comparing low- and high-level infestations separately, beetle emergence ranged from 1.2% to slightly over 7.2% for isoline corn, while MON 863 beetle emergence ranged from 0.006% to 0.145%. All categories had a female bias with MON 863 showing the greatest bias for beetle emergence. General conclusions were that the factors of treatment, environment, and treatment plus environment played significant roles in the selection intensity. It is recognized that interpreting data from selection studies from single locations or narrow geographies can be misleading; thus, adult emergence data may need to be collected on a greater scale from various geographies. Further types of similar analysis may need to be conducted to better understand the factors that may influence selection intensity.

A third study was conducted to initiate development of a MON 863-resistant colony (conducted by T. Clark, B. Hibbard, and I. Oyediran, Department of Entomology, University of Missouri). As of the 2004 progress report, four nondiapausing colonies, stemming from one wild-type x nondiapausing colony, were being reared and fed optimally as adults but differing in larval diet: 0) non-*Bt* (isoline) only, 1) exposed to *Bt* corn as neonates but reared on isoline, 2) *Bt* corn only from second instar to pupation and 3) reared solely on *Bt* corn. Numbering started at 0 to reflect the amount of exposure to Cry3Bb1-

expressing corn. Virgin female wild-type insects were crossed with nondiapausing males and virgin female nondiapausing beetles were crossed with wild-type males. From the resulting eggs (combined reciprocal crosses), a total of 4,262 F_1 beetles emerged that produced 241,000 eggs. From these eggs, colonies 0 through 3 were initiated and reared in the greenhouse. Results (as of 2004) showed that significant numbers of adults emerged with colonies 0 and 3 producing the most adults. Time from egg hatch to first adult was 27 days for colonies 0, 1, and 2 and 31 days for colony 3. The goal was to rear at least four to five generations of each colony per year to reach 10 generations. Generations 3, 6, and 10 were to be broadly tested for mechanisms of survival. The nondiapausing trait appeared to be completely dominant in the wild-type x nondiapausing colony that was created.

In separate research (not part of the MON 863 report), Miehls *et al*. (2008) were able to select for Cry3Bb1 resistance within three generations in greenhouse experiments. This work also suggested that the resistance trait could have non-recessive inheritance and could lead to rapid response to selection without adequate refuge.

iii. Dose

Identifying the level of dose, as related to selection intensity, is crucial when determining size and structure of a refuge needed to delay CRW resistance to MON 863 corn. CRW feeding behavior and survival and root expression data can be used to identify the dose of MON 863. From data currently available, it can be concluded that MON 863 corn does not provide a high dose for CRW control. The August 2002 SAP suggested that it is not necessary to determine the difference between a low and moderate dose. It is adequate to differentiate between high dose and non-high dose products when determining effective refuge size. Therefore, MON 863 should be characterized as a non-high dose product.

According to the August 2002 SAP, comparing measures of fitness levels of susceptible homozygotes on MON 863 and non-*Bt* corn would provide a good approximation of selection intensity. The SAP suggested that the first step in approximating selection intensity would be to measure efficacy of MON 863 corn against CRW larvae. The Panel pointed out, however, that selection intensity based on larval efficacy may be underestimated if sublethal effects or fitness costs occur. Selection intensity based on larval survival may also be underestimated if density-dependent mortality is occurring. Resistant colonies of CRW should be developed to aid in determining selection intensity.

The SAP based their determination that MON 863 is a non-high dose product on the SS (homozygous susceptible) survival rate. The Panel also concluded that Monsanto's artificial diet assays had deficiencies but were adequate to determine the median lethal concentration (LC_{50}) for first instar larvae, level of larval resistance, and dose.

iv. Simulation Models of Resistance

In Monsanto's three-year interim IRM plan, they recommended planting a 20% non-*Bt* corn refuge to delay the potential of CRW resistance to Cry3Bb1. Monsanto's conclusion that a 20% refuge would be

adequate to delay resistance to MON 863 corn was based on CRW biology, Cry3Bb1 effective dose, preliminary modeling results, and agronomic considerations. The Agency concluded that a 20% non-*Bt* corn refuge planted within or adjacent to MON 863 corn fields is expected to adequately delay the risk of CRW developing resistance to Cry3Bb1 (U.S. EPA 2002a). Monsanto's IRM interim plan and EPA's review of Monsanto's plan were addressed by the August 2002 SAP (U.S. EPA 2002b).

According to the SAP, the current models (Monsanto's modified Caprio model; Onstad *et al*. 2001; Andow and Alstad 2002) show that the time to resistance does not substantially differ when the refuge size ranges from 10–25%. While the SAP agreed that resistance would not occur during an initial 3 years regardless of the size of the refuge, the majority of the Panel recommended a 50% refuge would be a desirable conservative approach since resistance would be delayed substantially longer. The SAP also stated that the amount of gene frequency increases during an interim period is of greater importance than years to resistance because of the potential future impact on IRM. Since MON 863 is a non-high dose product, the Panel suggested that the potential for heritable quantitative variation and rapid evolution of resistance should be considered. In addition, the models only consider monogenic (single locus) resistance, but the SAP suggested that the models consider the potential for polygenic resistance in a non-high dose product.

Additional comments were made by the Panel regarding initial resistance allele frequency. Each of the models (Monsanto's modified Caprio model; Onstad *et al*. 2001; Andow and Alstad 2002) submitted in support of Monsanto's IRM plan designated the initial resistance allele frequency as 0.001. The Panel suggested that the initial resistance allele frequency may be as low as 0.1 in a non-high dose product. Therefore, the Panel recommended that studies be conducted to determine if the initial resistance allele frequency is less than 0.01, and models should be run that investigate the full range of dominance values.

In a letter dated December 13, 2002, Monsanto responded to the August 2002 SAP (Ward 2002). Within the letter, Monsanto summarized results from four data sets from research they sponsored on the efficacy of MON 863. The first and third data sets consisted of field data collected from 1999 to 2002 by 22 scientists from 15 universities located in 15 states. The second data set included data collected by Dr. Bruce Hibbard (University of Missouri), and the fourth data set was from research conducted by Dr. Blair Siegfried (University of Nebraska). According to Monsanto, results of these four data sets demonstrate that the initial allele frequency is ≤0.01. Detailed summaries of these four data sets will be submitted to the Agency for confirmation.

The first data set looked at 7,500 corn plants artificially infested with ≥1,200 CRW eggs/plant from naturally occurring populations. If the initial resistance allele frequency is 0.01 and Hardy-Weinberg is assumed, then 24 CRW/plant ($24 = 1{,}200(1-(1-0.01)^2)$) would be resistant, and the damage rating on the Iowa scale would be 3.1. Weiss *et al*. (1985) showed that <20 CRW = 3.1 on the Iowa scale. Since the average damage recorded in the first data set was 1.6, it can be concluded that the initial resistance allele frequency is ≤0.01.

The second data set summarized by Monsanto evaluated larval survival. In this study, ≥30 larvae were recovered per non-*Bt* corn plant at a wide range of egg infestation rates. If the initial resistance allele frequency is 0.01 and Hardy-Weinberg is assumed, then 0.6 resistant larvae ($0.6 = 30(1-(1-0.01)^2)$) would occur per MON 863 corn plant. Since an average of 0.7 larvae were recovered (but not feeding normally), a ≤0.01 initial resistance allele frequency can be assumed.

The third data set evaluated the number of surviving adult CRW. This data set included several studies that infest corn plants with over 1,200 eggs. Of the 1,200 eggs, an average of 30 adults survived on non-transgenic corn. If the initial resistance allele frequency is 0.01 and Hardy-Weinberg is assumed, then 0.6 resistant adults ($0.6 = 30(1-(1-0.01)^2)$) would occur per MON 863 plant, and the damage rating on the Iowa scale would equal 3. Since damage averages 1.6 on the Iowa scale, a ≤0.01 initial resistance allele frequency can be assumed.

The final data set (#4) examined 11 field-collected adult female CRW populations reared in the lab. Between 134 and 489 larvae per population were examined for susceptibility to Cry3Bb1. These larvae demonstrated less than 6-fold difference between the most and least susceptible populations, which is similar to or less than populations of European corn borer and corn earworm in their susceptibilities to Cry1Ac and Cry1Ab. If the initial resistance allele frequency is 0.01 and Hardy-Weinberg is assumed, then 2% (>20) of the larvae assayed would be resistant. Monsanto asserted that no putatively resistant large larvae were recovered at high doses, suggesting no larvae survived and there was low variation (lower than with lepidopterans); therefore, a ≤0.01 initial resistance allele frequency can be assumed.

Products with a resistance allele frequency ≥0.01 would not have enough efficacy to justify commercialization (Bourguet *et al*. 2003; Ferré and Van Rie 2002). If the initial resistance allele frequency were 0.1, then the efficacy of MON 863 corn would be so poor that it would not be a marketable product. At initial resistance allele frequencies of 0.1 and 0.01, damage would be greater than 4.6 and equal to 3 on the Iowa scale, respectively. The economic threshold in corn is a 3 on the Iowa scale. Monsanto has demonstrated that the average damage rating is 1.6. Since MON 863 consistently provides enough protection to result in much less than a 3 root rating, it can be concluded that the initial resistance allele frequency is ≤0.01 based upon product performance.

Monsanto modified Caprio's model to include an initial resistance allele frequency of 0.01 and submitted these results to EPA (Ward 2002). Results of running this model showed that a 20% refuge would delay resistance for approximately 7–16 years (Figure 2 on page 13 and Figure 3 on page 14 in Ward (2002)). For this model, SS survival was set at 0.5, and RS survival was set at 0.8, which is partial dominance. Based on data collected by Monsanto and its cooperators, MON 863 has been shown to control an average of 50% of the homozygous susceptible (SS) CRW. Therefore, the SS survival was designated 0.5 in the modified Caprio model.

According to Monsanto, RS survival (dominance) probably equals 0.7. Therefore, basing dominance on ≥0.8 would be considered a very conservative approach. Monsanto modeled RS survival to range from 0.5 to 1. If a RS survival of 1 (absolute worst case) were to occur and the initial resistance allele frequency is assumed to be 0.01, then resistance would be delayed for approximately 13 years with a

20% refuge (Figure 1 on page 8 in Ward (2002)). If RS survival is designated 0.8, then resistance will occur in approximately 16 years. According to Dr. David Andow (University of Minnesota), RS survival ranges between 0.3 and 0.8 (Andow, personal communication, 2002). Therefore, a likely case assessment would be to designate RS as 0.8, which suggests that 80% of the heterozygotes survive.

Monsanto also provided the Agency with additional runs of the modified Caprio model that included conservative parameters representing a worst-case scenario. These additional models included initial resistance allele frequencies of 0.01 and 0.001, RS dominance values of 0.7 and 0.8, and SS survival ranging from 0.1 to 0.8. Results of the model, incorporating these conservative input parameters (e.g., initial allele frequency = 0.01; RS dominance value = 0.8; SS survival = 0.1), suggested that CRW resistance to Cry3Bb1 will not occur for at least 7 years assuming 100% MON 863 market penetration and 100% IRM compliance (Table 1).

Table 1. Predictions for MON 863 Durability with a 20% Refuge.

SS Survival	RS Dominance	Allele Frequency = 0.01	Allele Frequency = 0.001
0.1	0.7	7 years	9 years
0.1	0.8	7 years	9 years
0.3	0.7	11 years	15 years
0.3	0.8	10 years	13 years
0.5	0.7	20 years	30 years
0.5	0.8	16 years	23 years
0.8	0.7	>100 years	>100 years
0.8	0.8	>50 years	>50 years

Monsanto also commented on the SAP's recommendation to consider polygenic resistance in the simulation models. According to Monsanto, results of the model will not differ if polygenic resistance is considered rather than monogenic resistance. Dr. Mike Caprio (Mississippi State University) agreed with Monsanto's conclusion. According to Dr. Caprio, applying monogenic or polygenic resistance to the models does not affect the outcome in the absence of refuge (Caprio 1998; Caprio, personal communication, 2002). Groeters and Tabashnik (2000) concluded "that the intensity of selection, rather than the number of loci conferring resistance, is central in determining rates of resistance evolution and effectiveness of refuges." This new information, provided to the Agency by Monsanto after the August 2002 SAP, suggests that assuming CRW resistance to MON 863 is polygenic rather than monogenic will not affect the results of the models.

Based on the additional information submitted by Monsanto after the August 2002 SAP and results of running Caprio's modified model with a 0.01 initial resistance allele frequency, it can be concluded that a 20% refuge will delay resistance for approximately 7 to 16 years and probably longer since the model also assumes 100% adoption. However, Monsanto assumes that 50% of the susceptible homozygotes

(SS) will be controlled. Efficacy data submitted thus far shows 17% to 62% larval survival on MON 863 corn. If the SS input parameter were changed to a lower level of efficacy (e.g., 0.3), then the years to resistance may decrease.

Based on the results presented in Monsanto's submission and recommendations from national experts, including the NCR-46, a 20% refuge should be adequate to delay resistance for 7 to 16 years. In addition, because growers are familiar with the 20% refuge required for currently registered *Bt* corn products, better compliance can be expected based on grower familiarity, feasibility, and presenting a consistent message to growers; a 20% refuge should be planted adjacent to or within fields.

v. Resistance Allele Frequency Data (MRID No. 459438-01)

Greenhouse and field efficacy studies, adult emergence trials, and laboratory feeding studies have generated data for estimating initial resistance allele frequency in corn rootworm populations feeding on transgenic MON 863 corn containing the genes for Cry3Bb1 endotoxin, as compared to isoline corn without Cry3Bb1 expression. These four studies (root damage ratings in the greenhouse and field; larval establishment in the field; adult survival in the field; and larval susceptibility to Cry3Bb1 in a laboratory bioassay) were conducted between 2000 and 2002. Monsanto and university trials, the work of Hibbard *et al*., and data from Siegfried and Spencer were summarized in this report. Artificial infestations (eggs) of WCRW were used to challenge both greenhouse and field populations of MON 863. Bioassay of artificial diet, top-loaded with five concentrations of purified Cry3Bb1 protein, and control were tested with larvae reared from eggs laid by adult female WCRW collected from 11 distinct field populations in six Midwestern states. Bioassay results for the MON 863 low-to-moderate dose product exhibited a 6-fold regional variation in larval susceptibility on the basis of LC_{50} values (2.22 micrograms per square centimeter ($\mu g/cm^2$) vs. 13.00 $\mu g/cm^2$). In all four studies, predicted results, based on the Hardy-Weinberg law and the assumption of an r-allele frequency of either 0.1 or 0.01 (for parameters of damage to MON 863 plants, number of larvae on each MON 863 plant, and number of resistant larvae), were compared with measured observations.

The evidence presented in the four data sets is largely circumstantial—r-allele frequency was estimated using three field efficacy studies and the results from Cry3Bb1 baseline susceptibility work. Major assumptions were made regarding density-dependent mortality, the genetics of potential resistance (assumed to be dominant), and the mode of action of MON 863 corn. While these indirect approaches provided some support to Monsanto's hypothesis that the r-allele frequency for CRW is <0.01, there was too much uncertainty to definitively prove that this was the case. In addition, there is still significant uncertainty regarding the mode of action of MON 863 (Cry3Bb1) corn and the nature of potential CRW resistance (U.S. EPA 2004d).

Monsanto has been required to continue to pursue the r-allele issue through the development of resistant CRW colonies (required as a condition of registration) and other research efforts. A better understanding of the mode of action (i.e., toxic or repellent effects) would also aid in the understanding of potential CRW resistance. In addition, research on dose, fitness, behavior, and possible polygenic inheritance could also be useful to further the understanding of CRW resistance.

vi. Refuge

A 20% non-*Bt* corn refuge is necessary to produce an adequate number of CRW susceptible to the Cry3Bb1 protein. There are two ways a grower can implement the refuge requirement. Non-*Bt* corn refuge can be planted as a continuous block adjacent to the MON 863 field or as non-transgenic strips planted within the MON 863 field. Considering the limited movement of CRW larvae, planting refuges close to transgenic fields in large blocks is preferred to narrow strips (Gray 1999; Meinke *et al*. 2001). If the 20% refuge is planted as row strips within a corn field, the non-*Bt* corn strips must be at least four or more consecutive rows wide. Use of an in-field strip refuge is not intended for fields planted to increase inbred seed since these fields need to be isolated from external corn pollen sources. An in-field or adjacent non-*Bt* corn refuge would be inconsistent with inbred seed production practices.

MON 863 corn was originally registered with a row width requirement of at least six rows for in-field strip refuges. Monsanto amended the terms of registration in 2005 to modify this requirement to at least four or more consecutive rows. This amendment was supported by larval movement data published by Hibbard *et al*. (2003) that showed between 0.75% and 6% of larvae moved across rows. This likely represents a relatively high-end estimate of the number of larvae that cross rows. Given this finding, narrower in-field strips should be sufficient to provide adequate protection from sublethal selection caused by CRW larval movement across rows. In addition, in-field strips of four or more rows for CRW provide the advantage of compatibility with the in-field strip width requirement for lepidopteran-protected *Bt* corn plant-incorporated protectants (PIPs) (also ≥4 rows), possibly increasing refuge compliance (U.S. EPA 2005d).

Insecticides, applied to the soil to control CRW larvae, are acceptable on refuge acres. The ability to treat refuges with larval insecticides is necessary to avoid the potential for severe damage and economic impact. It is not acceptable, however, to treat refuges for adult CRW control since these treatments may diminish the effectiveness of the refuge. If growers spray their corn fields with insecticides to control pests other than CRW, then all acres (*Bt* and non-*Bt*) should be treated identically.

Bt fields and non-*Bt* refuge acres should be treated with identical agronomic practices, such as irrigating all corn (*Bt* and non-*Bt*) at the same time. To ensure the production of similar numbers of CRW, *Bt* and non-*Bt* corn should be planted in fields with similar backgrounds. For example, if MON 863 hybrids are planted on continuous corn fields, then the non-*Bt* refuge should be planted on continuous corn fields or both should be planted on first-year corn acres. Likewise, non-*Bt* refuges should be planted on first-year corn fields if the MON 863 hybrids are planted on first-year corn fields.

vii. Monitoring for Resistance

A resistance monitoring strategy for *Bt* corn is needed to test the effectiveness of the resistance management programs. Detecting shifts in the frequency of resistance genes (i.e., susceptibility changes) through resistance monitoring can be an aggressive method to detect the onset of resistance before

widespread crop failure occurs. As such, the utilization of sensitive and effective resistance monitoring techniques is critical to the success of an IRM plan. Monitoring techniques, such as discriminating dose concentration assays, need to be thoroughly investigated for *Diabrotica* spp. for their feasibility as resistance monitoring tools.

Grower participation (e.g., reports of unexpected damage) is an important step in resistance monitoring. Resistance monitoring is also important because it provides validation of biological parameters used in models. Resistance detection/monitoring, however, is a difficult and imprecise task. It requires both high sensitivity and accuracy. Good resistance monitoring should have well-established baseline susceptibility data so changes in pest susceptibility over time can be monitored.

The August 2002 SAP recommended a two-tiered approach to monitoring for CRW resistance to MON 863. The Panel recommended tier 1 monitoring methods should identify locations that would merit tier 2 laboratory bioassays. Early detection monitoring should be directed to areas with the highest rate of MON 863 adoption since these areas represent the highest risk of resistance occurring.

The SAP suggested that current methods used for early detection of resistance probably do not have the necessary level of sensitivity. Therefore, the Panel recommended potential alternatives to the insect bioassay using artificial diet. For instance, susceptibility of neonate larvae to corn lines expressing varying levels of the Cry3Bb1 protein (e.g., Events MON 863, MON 862, MON 853, and MON 854) could be explored. Measuring larval mortality and growth data with various corn lines rather than artificial diet would be easier and may eliminate some of the problems associated with the feeding bioassay, such as mold growth on the artificial diet. Susceptibility data should also be collected for the NCRW and MCRW.

The SAP also suggested that data on root damage may be used as a monitoring tool; however, a method of using root damage ratings to monitor for resistance has not been developed or validated at this time. It also may be possible to use data on emergence patterns in the MON 863 and non-*Bt* corn refuges. More females than males from susceptible populations tend to emerge from MON 863. It may be possible to evaluate the percentage of males emerging and be correlated with resistance.

Monitoring will become more important after the accrual of multiple growing seasons of exposure and grower adoption increases. In addition to baseline susceptibility data, information is needed to determine how many individuals need to be sampled and in how many locations. The chance of finding a resistant larva in a *Bt* crop depends on the level of pest pressure, the frequency of resistant individuals, the location and number of samples that are collected, and the sensitivity of the detection technique. Therefore, as the frequency of resistant individuals or the number of collected samples increases, the likelihood of locating a resistant individual increases (Roush and Miller 1986). If the phenotypic frequency of resistance is 1 in 1,000, then more than 3,000 individuals must be sampled to have a 95% probability of one resistant individual (Roush and Miller 1986).

Cry3Bb1 Monitoring Strategy

As a condition of registration, Monsanto was required to develop a resistance monitoring program for Cry3Bb1 in MON 863 corn. The following elements were required for the plan:

- CRW sampling should be focused in those areas in which there is the highest risk of resistance development.
- The registrant must follow up on grower, extension specialist, or consultant reports of less than expected results or control failures for the corn rootworm.
- Baseline susceptibility studies for WCRW and NCRW and the investigation of techniques, such as discriminating dose concentration assays, for feasibility as resistance monitoring tools.
- Develop and validate an appropriate discriminating or diagnostic dose assay (required for MON 88017, a subsequent Cry3Bb1 registration).
- Rootworm damage guidelines for unexpected pest damage (required for MON 88017, a subsequent Cry3Bb1 registration).

Monsanto submitted a resistance monitoring strategy for MON 863 and Cry3Bb1 that was largely based on the established paradigm for lepidopteran *Bt* corn insects (reviewed in U.S. EPA 2004b). This monitoring plan was subsequently revised with the registration of MON 88017 (which also expresses Cry3Bb1) in 2005. The Cry3Bb1 monitoring plan (as developed for MON 88017; MRID No. 473547-01) is described below (reviewed in U.S. EPA (2009b and 2010)) and is applicable to MON 863 corn.

Monitoring for CRW resistance consists of two main parts: (1) monitoring for unexpected field damage by growers, extension agents, consultants, and company agronomists, and (2) monitoring for resistance through targeted population sampling and testing. Monitoring for unexpected damage will reveal the occurrence of localized resistance (or hot spots) before resistance will have spread. Resistance monitoring through targeted field sampling should reveal changes in susceptibility of geographically representative populations.

Population sampling will focus on the WCRW species, which will serve as a worst-case surrogate for northern corn rootworm and Mexican corn rootworm. Because of their widespread distribution and abundance, but similarity in life cycles compared to NCRW and MCRW, it is more likely that resistance due to exposure to Cry3Bb1 will evolve in WCRW first.

Monsanto proposed to focus its geographic sampling in areas where MON 863 and MON 88017 adoption has been highest and selection pressure is greatest. These areas are as follows:

Region 1, includes rotation-resistant variant
- Eastern Illinois
- Western Indiana

Region 2, wild-type variant
- Western Illinois
- Iowa
- Missouri

Region 3, organophosphate-resistant variant
- Nebraska
- Kansas

The breakdown into these three regions has been determined based on the three WCRW biotypes found in the U.S.: soybean/corn rotation-resistant, wild-type, and organophosphate-resistant. Monsanto proposed to target between 4–6 populations, but no less than 3, in these areas with different biotypes. Also, not all states specifically listed above may be represented by the sample collection. Actual sample sites are decided by DM Crop Research Group based on beetle abundance and environmental conditions. A periodic review of sales information may warrant modification of sample areas, though the Agency will be informed of such changes.

Population susceptibility will be assessed using a diet-based bioassays approach as described by Siegfried *et al*. (2005). The dose-response curve will be determined for each population and compared to historical data from populations in the same regions. A diagnostic concentration for Cry3Bb1 should be established using baseline susceptibility and annual monitoring data, as well as other historical information for WCRW and Cry3Bb1. Once a diagnostic concentration has been established, approximately 400 neonates (4 replicates total) will be tested at the assumed discriminating concentration.

Unexpected survivors at the discriminating dose will be reared to adults and mated amongst themselves or single-pair mated with individuals from a susceptible lab colony if numbers are low. The resulting progeny will once more be exposed to the diagnostic concentration bioassays to determine heritability of survival on a Cry3Bb1-containing diet. If heritability is confirmed, survivors will be placed on MON 88017 corn plants to assess whether level of resistance is enough to cause severe root damage. Whether an increase in susceptibility has occurred will be assessed with a discriminating concentration bioassay when such dose has been established. Until then, either of the following criteria below will serve to confirm resistance; however, Monsanto stated that their working definition of resistance will be refined based upon continued research and experience:

- The LC_{50} of the standard bioassay exceeds the 95% confidence interval of the mean historical LC_{50} for susceptible pests according to the baseline measurements.
- Over 50% of Cry3Bb1-expressing plants have ≥1 root nodes destroyed by suspected resistant populations under controlled lab conditions.

Unexpected Pest Damage

Since Cry3Bb1, as expressed in MON 88017, is not high dose, it will not control CRW at the same level as registered Lepidoptera protected *Bt* corn products. Hence, this requires a different approach to discern between unexpected pest damage and damage caused by CRW due to non-high dose control. Monsanto reported (MRID No. 473547-01) that the 2002 SAP Advisory Panel suggested consideration of the following factors specifically related to CRW resistance monitoring:

- CRW survival and some degree of root damage are expected in fields planted with MON 88017.
- A single corn root system supports numerous rootworm larvae. Therefore, the effect of resistant individuals on the overall root structure will not be easy to detect unless the resistant individuals represent a significant proportion of the population on that root system.
- Root damage caused by corn rootworm larvae feeding is not readily visible; plants must be dug up, and roots washed to assess damage.
- Aboveground symptoms of root damage, such as lodging, often have causes other than larval feeding (e.g., high winds in combination with high soil moisture content).
- Environmental factors can be significant determinants of the amount of damage caused by rootworm larvae.

Monsanto proposed final root damage guidelines of unexpected damage reports in a letter to EPA (dated January 20, 2010). The guidelines were submitted for MON 88017 (a subsequently registered plant-incorporated protectant (PIP) that expresses Cry3Bb1) but are also applicable to MON 863. The letter defined unexpected damage as: "[t]he level of root damage in MON 88017 field must be equal to or greater than that in the refuge field, assuming the MON 88017 and refuge fields are comparable with respect to management practices and the damage in the refuge is above 1.5 on the 0–3 nodal injury scale (NIS). In circumstances when a comparable refuge field is not available (e.g., refuge flooded, etc.), then guidelines for establishing suspect resistance are as follows:

1) Average root damage in the MON 88017 or MON 88017 x MON 810 field is >1.5 on the 0–3 NIS; and
2) The frequency of MON 88017 or MON 88017 x MON810 corn plants with >1.5 nodes destroyed exceeds 50% of the sampled plants."

Monsanto stated that these guidelines reflected the empirical experience of four years of CRW monitoring, which have allowed Monsanto to quantify the maximum amount of damage that could be expected under heavy infestation and favorable environmental conditions for rootworm feeding. This cumulative experience has led to an increase in the threshold NIS from the originally proposed 1.0 to 1.5

on the nodal injury scale. With the new threshold, Monsanto has found that a few false positive events still could be expected, making these guidelines a conservative choice.

Monsanto had previously indicated (MRID No. 473547-01) that if the above conditions were met and complaints of unexpected pest damage were received from the same growers in two consecutive years, they would attempt to collect CRW populations from the fields the following year. Collections would be undertaken when CRW flight is at its peak and approximately ½ mile away from the nearest MON 88017 fields.

EPA's review of Monsanto's unexpected damage strategy identified a number of concerns with the proposed approach (U.S. EPA 2010a). A damage threshold level of 1.0 may be more appropriate and conservative for moderate insect pressure and single, non-high dose CRW products, such as MON 88017 and MON 863. The review recommended that Monsanto keep the threshold level for unexpected pest damage at 1.0 when insect pressure is low to moderate. When CRW pressure is exceedingly high during a corn-growing season, however, then *Bt*-protected and refuge corn will likely incur greater damage. Under these circumstances, a threshold level of 1.5 may be more appropriate as proposed by Monsanto. The review also recommended consideration of the following factors for unexpected pest damage:

1) The inherent dose of the toxin to control CRW (high dose vs. non-high dose control);
2) Prior use and crop history in the *Bt* field where excessive damage was observed;
3) Damage on non-*Bt* plants in the same field or immediately adjacent to the *Bt* plants;
4) Insect pressure during that corn-growing season (moderate vs. high); and
5) Weather pattern during the corn-growing season and possible effects on *Bt* protein expression and pest population dynamics.

Further, concerns were identified with Monsanto's approach to respond to unexpected damage only after reports have been received in two consecutive years by the same grower. This approach may be too protracted (two years before sample collection occurs and four to five years total before resistance is confirmed) and could lead to the undetected spread of resistance in that region. It was recommended that Monsanto respond to reports of unexpected pest damage during the same growing season when the report has been filed, or no later than July of the following growing season (U.S. EPA 2009b and 2010).

Baseline Susceptibility of Corn Rootworm to Cry3Bb1

As required by the terms and conditions of the MON 863 registration, baseline susceptibility levels of western corn rootworm populations to Cry3Bb1 have been measured (Siegfried *et al*. 2005). These data serve as the baseline for measuring any annual shifts in the susceptibility of these populations. Results indicated that the representative WCRW populations collected in 2004 were susceptible to the Cry3Bb1 toxin and that slight differences in susceptibility among the populations were due to natural variation in responses. In addition to the baseline susceptibility studies conducted on western corn rootworm, populations of the northern corn rootworm and Mexican corn rootworm were being collected as part of the required monitoring program and to determine baseline susceptibility levels for these species.

Improved methods for collecting Mexican corn rootworm adults have been published (Spurgeon *et al*. 2004) and may help in this on-going effort to monitor rootworm populations.

EPA reviewed (U.S. EPA 2006a) the baseline monitoring data provided by Monsanto from 2001–2004 (no data available for 2003) and concluded that all representative WCRW populations were susceptible to the Cry3Bb1 toxin (expressed in MON 863).

Cry3Bb1 Discriminating (Diagnostic) Concentration for CRW

As a condition of registration, Monsanto was required to investigate the feasibility of employing a discriminating (or diagnostic) concentration assay for CRW monitoring. Diagnostic concentration assays function by identifying potential resistant individuals that can survive high levels of the controlling toxin. For lepidopteran target pests of other *Bt* corn PIPs, these assays have been based on the LC_{99} of susceptible populations.

Monsanto developed a diagnostic concentration for Cry3Bb1 of 170.8 $\mu g/cm^2$ against WCRW (MRID No. 478846-01). Low and variable survival was still apparent at this level; however, surviving larvae were severely stunted as observed in field-collected populations of 2007 and 2008. Table 2 summarizes the percent mortality and mean mass of survivors of field populations collected during the two years and compares the results to those obtained from the susceptible lab reference strain. The weight of larval survivors from field populations ranged from 0.01–0.04 mg compared to the mean mass of control larvae (0.22 mg). Monsanto concluded that this concentration appeared to fit the needs for an effective diagnostic concentration where rootworm response is measured as larval mass at the end of the assay. Should field-derived survivors show significant development, Monsanto would further investigate this population for potential resistance to Cry3Bb1.

Table 2. Percent Mortality and Mass of Surviving Larvae at the Putative Diagnostic Concentration of 170 µg/cm^2.

Population	Average % mortality across replicates	Average mass of survivors (mg) across replicates	Maximum mass (mg) across replicates
Montgomery Co., IN	77.8	0.02	0.04
McLean Co., IL	85.4	0.01	0.02
Dewitt Co., IL	88.9	0.01	0.02
Scott Co., IA	86.1	0.01	0.02
York Co., NE	86.1	0.01	0.02
Polk Co., NE	73.6	0.02	0.03
Seward Co., NE	47.2	0.03	0.05
Piatt Co., IN	75.0	0.02	0.03
Henry Co., IL	44.4	0.03	0.05
Palo Alto Co., IA	54.2	0.04	0.05
Logan Co, NE	48.6	0.03	0.04
Clinton Co., IA	76.4	0.02	0.03
Monsanto Lab	62.5	0.04	0.07
Population Range	**44.4–88.9**	0.01–0.04	0.02–0.07
Field population control – No Cry3Bb1 exposure	**Average % mortality across replicates**	**Average mass of survivors (mg) across replicates**	**Maximum mass (mg) across replicates**
All sites	13.3	0.22	0.41

*Table data extracted from MRID No. 478846-01.

EPA has also recommended that Monsanto investigate the sublethal seedling assay (SSA) developed by Nowatzki *et al*. (2008), in addition to their effective diagnostic bioassays, to determine which approach is more sensitive to detect shifts in CRW susceptibility. Nowatzki *et al*. (2008) tested the sensitivity of SSA side-by-side with a diet bioassay. They found that the SSA, measuring survival and age structure of larval populations in three potential instar-groups, was able to detect shifts in susceptibility of CRW at a much smaller scale than the diet bioassay, which measured mortality and growth inhibition responses. Diet bioassay endpoints (LC_{50} and EC_{50}) were relatively insensitive to detecting shifts in susceptibility (treatments were 0%, 5%, 25%, and 50% selected individuals mixed into susceptible population samples of CRW), while the SSA was most sensitive to changes in susceptibility when selected individuals were present at $\leq$25%. Nowatzki *et al*. (2008) stated that the SSA may be a more sensitive tool to measure shifts in susceptibility than the bioassay because it uses the increased sensitivity of a sublethal measure (developmental shifts of larvae into three instar stages/cohorts).

Cry3Bb1 Resistance Monitoring Data

Resistance monitoring data for Cry3Bb1 and WCRW have been tabulated from 2005 through the 2008 growing season. For 2008 (MRID No. 478846-01), DM Crop Research Group (an independent party that collects corn pest samples) made thirteen field collections from areas of high CRW pressure and Cry3Bb1 adoption to represent populations with the highest potential risk of resistance evolution. Custom Bio-Products in Maxwell (IA), an independent laboratory that has conducted bioassays for Monsanto since 2006, maintained the insect collections and conducted the bioassays. Populations were

maintained using standard protocol for this species; in addition, a nondiapausing lab population was supplied by Monsanto as a reference. The form of Cry3Bb1 used was a solution of 4.1 milligrams per milliliter (mg/mL) supplied by Monsanto Company. This solution was diluted with 0.1% Titron-X 100 to obtain a series of concentrations of the Cry3Bb1 protein for bioassay. Neonate larvae were used for diet overlay bioassays and exposed to different Cry3Bb1 concentrations (10.7 µg/cm^2, 21.4 µg/cm^2, 42.7 µg/cm^2, 85.4 µg/cm^2, and 170.8 µg/cm^2). Thirteen micrograms of each dilution was applied to 12 individual wells and allowed to dry prior to larval introduction. Larvae were non-systematically selected and placed into wells of the tissue culture tray. After three to four days, mortality and survival was recorded. Six replicates were conducted for each field and the Monsanto lab population. A replication was considered valid if control mortality did not exceed 25%.

Observed EC_{50} values from the 2008 populations ranged from 7.3–30.4 µg/cm^2, representing a 4-fold difference in susceptibility, and were comparable to the EC_{50} range obtained in 2007 (14.2–33.4 µg/cm^2). The mean EC_{50} of the lab reference strain was comparable to those of most field populations as measured by the 95% confidence interval overlap. Based on the data, Monsanto concluded that the variation observed in EC values was due to the nature of the Cry3Bb1 protein standard rather than changes in susceptibility of field populations. LC_{50} values were more variable than EC values: (1) LC_{50} range: 24.5–335.0 µg/cm^2 and (2) LC_{90} range: 218.2–43,931.0 µg/cm^2. Higher LC values may be an artifact of the assay system because larvae are able to survive without feeding.

Several portions of the Corn Belt have been sampled consistently since 2005 (Scott Co., IA; Seward Co., NE; and Henry Co., IL). A comparison of the CRW susceptibility data in these geographic areas (summarized in Table 3 below) showed that susceptibility (as measured by EC_{50} and LC_{50}) appeared to decrease during the 2005–2008 sampling period. The susceptibility of the field population, however, has been comparable to the lab reference strain.

Table 3. CRW Susceptibility to Cry3Bb1 for Three Counties in the Corn Belt as Measured by EC_{50} and LC_{50} Data (2005–2008).

Population	Mean EC_{50} ($\mu g/cm^2$)	Mean LC_{50} ($\mu g/cm^2$)
Scott County, IA (2005)	0.3	0.5
Scott County, IA (2006)	1.7	6.6
Scott County, IA (2007)	15.5	63.8
Scott County, IA (2008)	25.8	40.0
Henry County, IL (2005)	1.9	3.2
Henry County, IL (2006)	1.9	5.6
Henry County, IL (2007)	16.2	50.2
Henry County, IL (2008)	14.8	300.9
Seward County, NE (2005)	2.6	3.3
Seward County, NE (2006)	1.3	9.3
Seward County, NE (2007)	14.2	64.2
Seward County, NE (2008)	9.4	335.0
Monsanto Reference Strain (2007)	***12.9***	**22.3**
Monsanto Reference Strain (2008)	***21.8***	**87.9**

***Table generated from data submitted by Monsanto.**

Susceptibility data (EC_{50}, LC_{50}, and LC_{90}) from population samples collected across the Corn Belt during 2005–2008 are summarized in Table 4 below. These data show that there has been no apparent increase in susceptibility in CRW across the Corn Belt and support Monsanto's conclusion that WCRW remain susceptible to the Cry3Bb1 protein.

Table 4. CRW Susceptibility to Cry3Bb1 - Data Ranges for All Populations Sampled in the Corn Belt (2005–2008).

Year	EC_{50} ($\mu g/cm^2$)	LC_{50} ($\mu g/cm^2$)	LC_{90} ($\mu g/cm^2$)
2005	0.25–1.28	0.42–1.36	
2006	0.64–1.88	1.43–22.22	
2007	12.91–33.46	22.29–289.25	97.03–1E+6
2008	7.3–30.4	24.5–335	218.2–43,931

***Table generated from data submitted by Monsanto.**

viii. Remedial Action

Background and Recommendations for Remedial Action

The initial observation of unexpected CRW damage or suspected resistance will likely occur by the grower. Unexpected damage will probably be observed as lodged corn plants in the fields. Growers should be required to report any unexpected CRW damage, such as lodged plants, to the registrant. The August 2002 SAP identified the following four steps a registrant should take to determine if further testing is needed to confirm resistance is occurring:

- "request the grower check planting records"
- "rule out damage from nontarget insects, weather, or other environmental factors"
- "conduct tests to verify MON 863 was planted and that the correct percentage of plants are expressing"
- "if plants are MON 863 and damage approaching a 0.5 (Node Injury Scale) is found on any expressing plant, evaluate roots from the corresponding refuge"

Resistance should be confirmed by a standard diet bioassay or evaluation of root node injury. An insect diet bioassay with the Cry3Bb1 protein that results in a LC_{50} that exceeds the upper limit of the 95% confidence interval of the LC_{50} established from baseline measurements of susceptible populations could be used to confirm resistance. Alternatively, resistance may be confirmed when one or more root nodes of at least 50% of Cry3Bb1 plants grown in the laboratory are destroyed. A discriminating concentration bioassay may also be used to confirm resistance; however, this method may take a long time to develop. The August 2002 SAP also recommended investigating the potential of using samples of populations surviving on *Bt* corn or an evaluation of larval root tunneling to confirm resistance.

Confirmed resistance should be reported to EPA as soon as possible but no later than 30 days. Once resistance has been confirmed, alternative control measures to reduce or control the local target pest population should be recommended to customers, extension agents, consultants, university cooperators, seed distributors, processors, state regulatory authorities, EPA regional and national authorities, and any other pertinent personnel of the incidence(s) of resistance in the affected area. Where appropriate, customers and extension agents in the affected area should apply insecticides and/or crop rotation practices to control any potentially resistant individuals.

As soon as possible following confirmation of resistance, but within 90 days, Monsanto should notify the Agency of the immediate mitigation measures that were implemented and submit a proposed long-term resistance management action plan for the affected area. Monsanto should work closely with the Agency in assuring that an appropriate long-term remedial action plan for the affected area is implemented. A remedial action plan that is approved by EPA should be implemented that consists of some or all the following elements, as warranted: (1) Inform customers and extension agents in the affected area of pest resistance; (2) Increase monitoring in the affected area, ensuring that local target pest populations are sampled on an annual basis; (3) Recommend alternative measures to reduce or

control target pest populations in the affected area; (4) Implement intensified local IRM measures in the affected area based on the latest research results (implementation of such measures will be coordinated by the Agency with other registrants); and (5) Monsanto should cease sales of all MON 863 *Bt* corn hybrids in the affected area until resistance has abated. During the sales suspension period, Monsanto may sell and distribute in these counties only after obtaining EPA approval to study resistance management in those counties. The implementation of such a strategy should be coordinated with the Agency.

For the growing season(s) following a confirmed resistance incident(s), Monsanto should maintain the sales and distribution suspension of all MON 863 hybrids potentially affected by the resistant pest populations and/or areas in which resistance is considered to be serious. This should be done within the affected region or if undetermined, the affected county(ies) and proximate surrounding counties. This sales suspension should remain in place until resistance has been determined to have subsided (within 5% to 10% or one standard deviation of baseline levels). In addition, Monsanto should develop, recommend, and implement alternative resistance management strategies for controlling the resistant pest(s) on corn with all necessary personnel (e.g., growers, extension agents, consultants, seed distributors, processors, university cooperators, and state/federal officials) in the affected region/county(ies) and surrounding counties of the resistance situation. All necessary personnel (e.g., growers, consultants, extension agents, seed distributors, processors, university cooperators, and state/federal authorities) in the affected region/county(ies) and surrounding counties of the resistance situation should be informed. Monitoring and surveillance in the affected area(s) for resistance and defining the boundaries of the affected region should be intensified, and studies on the rate of decline of resistance in the field should be conducted. Monsanto should continue to work with the Agency, states, grower groups, extension agents, consultants, university cooperators, or other expert personnel and stakeholders to ensure the implementation and development of appropriate mitigation measures for resistance in the affected areas.

Remedial Action Plan for MON 863 (Cry3Bb1 Corn)

As a term of registration, Monsanto was required to develop a remedial action plan for MON 863 corn to address many of the issues discussed above. Details of the process used to confirm resistance in suspected CRW populations are described in the resistance monitoring plan for Cry3Bb1 (submitted for MON 88017, MRID No. 473547-01). A stepwise remedial action plan was submitted separately by Monsanto to fulfill the terms of registration (submission dated January 22, 2004, No MRID No.).

As described in the resistance monitoring plan for Cry3Bb1, unexpected survivors in discriminating dose assays will be reared to adults and mated amongst themselves or single-pair mated with individuals from a susceptible lab colony if numbers are low. The resulting progeny will once more be exposed to the diagnostic concentration bioassays to determine heritability of survival on a Cry3Bb1-containing diet. If heritability is confirmed, survivors will be placed on Cry3Bb1 corn plants to assess whether level of resistance is enough to cause severe root damage. Whether an increase in susceptibility has occurred will be assessed with a discriminating concentration bioassay when such dose has been established.

Until then, either of the following criteria will serve to confirm resistance; however, Monsanto stated that their working definition of resistance will be refined based upon continued research and experience:

- The LC_{50} of the standard bioassay exceeds the 95% confidence interval of the mean historical LC_{50} for susceptible pests according to the baseline measurements.
- Over 50% of Cry3Bb1-expressing plants have ≥1 root nodes destroyed by suspected resistant populations under controlled lab conditions.

Monsanto proposed a 2.5-year timeframe from the moment of initial detection of CRW resistance to Cry3Bb1 to the actual implementation of an appropriate remediation plan. This is the same timeframe that has been proposed for lepidopteran resistance in *Bt* corn. Because PIPs containing Cry3Bb1 are not expressed at high dose levels, resistance is likely to be additive and potentially dominant. ABSTC (2003) demonstrated in their lepidopteran monitoring plan that if resistance is additive and dominant, detection needs to occur at resistance allele frequency levels ≤0.03 and 0.002, respectively, to allow detection 2.5 years before the population has become resistant. The sample size needed is 1,000 insects in order to detect a particular allele frequency with 80% or 95% confidence when resistance is incomplete or dominant. Failure to detect resistance with 1,000 genomes suggests that the resistance allele frequency is less than 0.001. The upper 80% and 95% confidence limits of this estimate (0.001) are 0.0016 and 0.003, respectively, which are compatible with the allele frequency detection thresholds (0.03 and 0.002) needed for a 2.5-year remedial action timeframe before resistance occurs.

Monsanto's proposed remedial action plan and steps are more suitable to situations where field resistance is detected through product performance monitoring and subsequently confirmed in the lab. These steps may include the following:

- Confirm that resistance is heritable;
- Confirm field resistance;
- Use crosses to determine the nature of resistance;
- Estimate the r-allele frequency in the original population;
- Determine whether the r-allele frequency is increasing by analyzing field collections;
- Sample from the site in subsequent years where the resistant allele(s) was originally collected and determine if resistance is still detectable;
- Determine the geographic distribution of the r-allele by analyzing field collections in subsequent years from sites surrounding the site where the resistant allele was originally collected;
- If the r-allele frequency is determined to be increasing or spreading, design an appropriate remedial action plan based on the knowledge of the genetics and level of resistance it confers in the field.

Monsanto's remedial action plan for MON 863 (as described in the January 22, 2004 submission) is as follows:

1. In cases of suspected resistance, the registrant will instruct growers to do one or more of the following:
    a. During the present season, use conventional insecticides to control the adult stage of the suspected pest;
    b. During the following season, use an alternative pest control method to deter establishment of potentially resistant insects.
2. If resistance is confirmed, Monsanto will:
    a. Increase resistance monitoring in the affected area.
    b. Notify affected growers, consultants, extension agents, seed distributors, university cooperators, and state/federal authorities of the resistance event.
    c. Instruct affected growers and extension agents to use alternative CRW control measures.
    d. Report the incident to EPA within 30 days of confirming pest resistance.
    e. Within 90 days of confirmed pest resistance:
        i. Notify the Agency of any mitigation measures that have been implemented;
        ii. Work with the Agency to develop an appropriate (in agreement with current research on IRM) resistance management action plan for the affected region;
        iii. Submit a proposed action plan to the Agency.
3. If resistance mitigation efforts (described above) fail, Monsanto will stop the sale and distribution of YieldGard® CRW in the remedial action zone (may be less than a single county, a single county, or multiple counties), until an effective mitigation plan has been approved by EPA.
4. The EPA-approved management plan for the affected area may consist of some or all of the following elements:
    a. Annual IRM education for relevant parties (e.g., growers, consultants, extension agents, seed distributors, university cooperators, and state/federal authorities).
    b. Annual resistance monitoring.
    c. Use of alternative pest control measures to reduce or control target pest populations.
    d. Development of alternative resistance management strategies.
    e. Suspension of YieldGard® CRW sales until an EPA-approved resistance management plan is in place.

EPA's review of the remedial action plan (U.S. EPA 2004b) found the framework of the plan to be acceptable.

ix. Compliance

Grower compliance with refuge and IRM requirements is a critical element for resistance management. Significant non-compliance with IRM among growers may increase the risk of resistance for Cry3Bb1 corn.

MON 863 Compliance Assurance Program

As a term of the MON 863 registration, Monsanto was required to develop and submit to EPA a compliance assurance program (CAP) to ensure grower adherence to IRM requirements. The terms of registration mandated a number of components for the compliance program including the following:

- Grower Agreements: Contractual arrangement between the registrant and grower to obligate adherence to IRM requirements.
- Annual IRM Survey: A survey (conducted anonymously by an independent research firm) intended to provide a statistically representative sample of growers from various corn-growing regions in the U.S. Results from the survey should assess levels of grower compliance with refuges as well as grower motivations, attitudes, and reasons for non-compliance.
- On-Farm Assessments: The registrant is required to develop an on-site assessment program in which trained personnel from each company make visits to farms growing *Bt* corn. During these visits, compliance with refuge requirements is assessed, and growers not in compliance are identified for corrective action under the Phased Compliance Approach.
- Tips and Complaints: The registrant must establish a means for the reporting and investigation of incidences of refuge non-compliance.
- Phased Compliance Approach (PCA): A consistent set of procedures (for all *Bt* corn registrants) to be employed to address non-compliance among growers and seed dealers.

The Agricultural Biotechnology Stewardship Technical Committee (ABSTC), a consortium of *Bt* corn registrants, previously developed and submitted a CAP for lepidopteran *Bt* corn PIPs in 2002. Subsequently, ABSTC submitted revised versions of the CAP in 2004 and 2005 in response to EPA reviews of annual growing season reports (see discussion in the U.S. EPA (2010b)). MON 863 (and other Cry3Bb1 registrations) have been included as part of this existing program, though data are tabulated separately for lepidopteran, rootworm, and stacked (lepidopteran + rootworm) *Bt* corn PIPs. EPA reviews of compliance data for rootworm-protected PIPs (described below) can be found in U.S. EPA (2004a, 2007, and 2009a).

Annual Grower Surveys

As a condition of each individual *Bt* corn registration, the registrant must perform an annual third-party survey of a statistically representative sample of *Bt* corn growers. The grower survey functions to measure compliance adherence to refuge size and distance requirements at a regional level and to identify educational opportunities to increase grower compliance with IRM requirements. More than 500 growers from 4 separate regions are anonymously surveyed annually. The methodology for conducting the grower survey has remained virtually unchanged since it was first conducted by Marketing Horizons, Incorporated in 2000 for the lepidopteran *Bt* corn registrations. Starting in 2007, however, due to an increasing complexity of growers' *Bt* corn planting practices and a need to standardize the grower survey across insect-protected traits, Marketing Horizons, Incorporated utilized an internet-based survey approach.

Surveys for the corn rootworm PIPs encompass all growers planting rootworm-protected traits (Cry3Bb1, mCry3A, and Cry34/35Ab1). Cumulative results of the surveys are summarized in Table 5 below. Results from the stacked (lepidopteran + rootworm) *Bt* corn surveys are tabulated separately (Table 6) and also include all registered rootworm PIP traits.

Table 5. Summary of Telephone (2005–2006) and Online (2007–2008) Survey Results for Rootworm-Protected *Bt* Corn Growers.

Survey Question	2005[1] % Respondents	2006[2] % Respondents	2007 % Respondents	2008 % Respondents
Adherence to Refuge[3] Size	93	89	80	74
Adherence to Distance Requirements[3]	87	82	79	63
Awareness of IRM Requirements	97	93	97	96
Unaided Recall of Refuge Size	51	57	63	72
Unaided Recall of Refuge Distance	58	55	33	34

[1] Includes YieldGard® RW and YieldGard® Plus corn growers
[2] Includes YieldGard® RW, YieldGard® Plus, Herculex® RW, and Herculex® XTRA corn growers
[3] Weighted averages across all four regions surveyed

Table 6. Summary of Telephone and Online Survey Results for Stacked *Bt* Corn Growers (2006–2008).

Survey Question	2006 % Respondents	2007 % Respondents	2008 % Respondents
Adherence to Refuge Size[1]	78	70	72[4]
Adherence to Distance Requirements[1]	92	66	66[3]
Awareness of IRM Requirements	95	96	97
Unaided Recall of Refuge Size	59	62 and 55[2]	81
Unaided Recall of Refuge Distance CRW	48	39	36
Unaided Recall of Refuge Distance European Corn Borer (ECB)	32	77	86

[1] Weighted average across all four regions surveyed
[2] First number listed is for ECB and the second number for CRW refuge compliance.
[3] On a per field basis, adherence was 76%.
[4] On a per field basis, adherence was 73%.

Overall compliance (per grower) with refuge requirements for both single-trait and stacked rootworm-protected PIPs has declined from 2005 to 2008. Grower adherence to the necessary refuge size fell to below 75% in 2008 for single-trait and stacked rootworm PIPs. Compliance with refuge proximity was lower; in 2008, ≤66% of rootworm PIP growers deployed refuges within the required distance to the *Bt* field. The percent of growers who were able to recall the correct refuge distance requirements (unaided) for rootworm PIPs drastically declined to below 40% in 2008. Refuge distance requirements for rootworm-protected *Bt* corn products may be more challenging for growers because the refuge must be deployed either within or immediately adjacent to the *Bt* field. Stacked products present additional challenges due to the need to plan either two refuges (for lepidoptera and rootworm) or a combined refuge for both pest complexes. Grower awareness of the distance requirements has been poor and likely explains much of the reported non-compliance.

On-Farm Assessments

The on-farm assessment program is the portion of the CAP that identifies individual non-compliant growers (regardless of farm size) for remedial IRM education, follow-up reassessments, and other activities as part of the PCA. It can also serve as a tool to enhance the registrant's understanding of the obstacles growers face in implementing IRM requirements. The mandatory on-farm assessment program was fully implemented for the first time in 2003 (for lepidopteran registrations) and has typically encompassed more than 2,000 growers per season (for all types of *Bt* corn). On-farm assessments for rootworm-protected PIPs (including Cry3Bb1 products) began in 2006.

Data from the on-farm assessments (2006 through 2008) of rootworm-protected *Bt* corn PIPs are

summarized in Table 7 below. These on-farm assessments encompass all growers planting rootworm-protected PIPs, including varieties expressing the Cry3Bb1, mCry3A, and Cry34/35Ab1 toxins. Results for the on-farm assessments of stacked (lepidopteran + rootworm) PIPs are detailed in Table 8. The assessments do not have the statistical power associated with the consistently stratified and randomized telephone/on-line surveys and are not used to measure representative rates of non-compliance. In 2007 and 2008, no information was provided regarding specific non-compliance with refuge size and distance. This information should be provided in future reports to be consistent with previously collected data and to illustrate how growers are out of compliance (U.S. EPA 2009a).

Table 7. Cumulative Results for the On-Farm Assessments of Coleopteran-Protected *Bt* Corn Growers (2006–2008)[1].

	2006	2007	2008
Growers Assessed	395	247	134
Refuge Distance Deviations[2]	13	N/A	N/A
Refuge Size Deviations	7	N/A	N/A
Significant Deviations	11 (2.8%)	16 (6.5%)	12 (9.0)
Insignificant Deviations	10 (4.0%)	8 (3.2%)	7 (5.2%)
Compliant Growers	374 (94.7%)	223 (90.3%)	115 (85.8%)
Non-Compliant Growers	21 (5.3%)	24 (9.7%)	19 (14.2%)

[1]Table adapted from page 12 of MRID No. 470444-01.
[2] Some growers had compliance deviations other than refuge size or distance; thus, the total of refuge distance and size deviations does not equal the number of non-compliant growers.

Table 8. Cumulative Results of the On-Farm Assessments of Stacked *Bt* Corn (Rootworm + Corn Borer) Growers (2006–2008)[1].

	2006	2007	2008
Growers Assessed	600	1069	1799
Refuge Distance Deviations	51[2]	N/A	N/A
Refuge Size Deviations	8	N/A	N/A
Significant Deviations	45 (7.5%)	77 (7.2%)	**86[3]**
Insignificant Deviations	16	33 (3.1%)	**36[3]**
Compliant Growers	539 (89.8%)	959 (89.7%)	1546 (85.9%)
Non-Compliant Growers	61 (10.2%)	110 (10.3%)	253 (14.1%)

[1] Table adapted from page 12 of MRID No. 470444-01.
[2] Some growers had compliance deviations other than refuge size or distance; thus, the total of refuge distance and size deviations does not equal the number of non-compliant growers.
[3] The numbers of deviations do not add up to the 253 non-compliant cases reported.

Tips and Complaints

As required by the terms of registration, *Bt* corn registrants must have a "tips and complaints" system as a mechanism for individuals (e.g., growers, sales representatives, etc.) to report alleged instances of IRM non-compliance. The number of tips and complaints (summarized for all *Bt* corn registrations, including lepidopteran and rootworm varieties) received through 2008 is summarized in Table 9 below. Each of these growers identified through the tips and complaints mechanism were visited as part of the on-farm assessment program. However, it is not possible to determine whether any of the non-compliant growers, identified via the tips and complaints route, were subject to the Phased Compliance Approach.

Table 9. Anonymous Tips and Complaints about Non-Compliance with IRM Requirements (Data from ABSTC Annual Reports 2003 through 2008).

Year	Number of Tips and Complaints
2003	0
2004	0
2005	5
2006	3
2007	14
2008	5

Phased Compliance Approach

ABSTC's CAP for lepidopteran- and rootworm-protected PIPs includes a standard set of procedures (shown in Table 10), known as the Phased Compliance Approach (PCA), which is to be used by registrants when responding to instances of grower non-compliance with the IRM requirements. The PCA also established a tiered approach for non-compliance with "significant" deviations and "other" deviations. For a 20% CRW refuge requirement (Corn Belt), a significant size deviation is defined as a *Bt* grower planting less than 15% non-*Bt* corn refuge. This definition is also applicable to "combined" refuges planted for both lepidoptera and CRW for stacked *Bt* corn PIPs. On the other hand, a significant deviation based on refuge proximity has not been clearly defined for CRW refuges and, as of the 2008 CAP report, it is unclear what standards are being used by ABSTC. For lepidopteran *Bt* corn, a significant deviation is triggered if fewer than 2/3 of the *Bt* corn fields are planted within ½ mile of a non-*Bt* corn refuge. However, this definition is not compatible with CRW refuge because the distance requirement mandates that refuges be placed adjacent to or within the *Bt* corn field. A reasonable extension of the lepidopteran definition for CRW could be "less than 2/3 of the non-*Bt* refuge is deployed adjacent or within the *Bt* field" and "fewer than two-thirds (2/3) of the in-field strips are at least four rows wide" (U.S. EPA 2007). This formula would also be applicable to combined refuges for stacked PIPs.

Table 10. Phased Compliance Approach (PCA) – Standards for *Bt* Corn Refuge Non-Compliance (Submitted with the ABSTC 2002 CAP).

	Mandatory Responses	Additional Responses
Significant Deviations	• IRM education • Warning letter • Compliance assistance contact prior to planting • Compliance assessment contact for the following growing season • Deny access to the *Bt* corn product for any significant deviation two years in a row.	• Invoice monitoring • Technical assistance • Grower IRM training • Reaffirmation of IRM obligations • Deny access to the *Bt* corn product for other deviations that are repeated over a period of years
Other Deviations	• IRM education • Letter and/or compliance assistance contact prior to planting • Compliance assessment contact in the following growing season	

Under the PCA, sales are to be suspended to individual growers for one year after two years of significant deviations. Following the one-year suspension, growers will need to be requalified to purchase seeds. Growers identified as non-compliant (significant or other deviations) are required to receive a "compliance assessment contact" the following year under the PCA. Non-compliant growers are typically identified through the on-farm assessment program (see previous discussion in the "On-Farm Assessments" section). Table 11 summarizes the numbers of non-compliant growers reassessed under the PCA, and the growers still found to be out of compliance. As of the 2008 growing season, one grower was denied access to *Bt* corn technology due to a refusal to be reassessed in the following season after significant non-compliance. Compliance data, including results of on-farm assessments and PCA activities, are detailed in U.S. EPA (2007 and 2009a).

Table 11. Reassessment of Rootworm-Protected and Stacked *Bt* Corn Growers Under the Phased Compliance Approach (Taken from ABSTC Annual CAP Reports)[1].

Year	Reassessments[2]	Significant Deviations[3]	Loss of Access to Technology
2006	62	0	1[4]
2007	82	0	0
2008	134	0	0

[1] The data in this table includes both growers planting single-trait rootworm PIPs and stacked (lepidopteran + rootworm) PIPs. The data in the table has been summed for both groups.
[2] Reassessments of growers identified with deviations (significant and other) to refuge requirements the previous growing season.
[3] Significant deviations recorded the following season. Two successive years of significant deviations results in loss of access to *Bt* corn technology.
[4] One grower refused to be reassessed in 2006 and was denied access to *Bt* corn.

x. Grower Education

Growers are perhaps the most essential element for the implementation and success of any IRM plan as they will ultimately be responsible for ensuring that refuges are planted according to guidelines and that *Bt* fields are monitored for unexpected pest damage. Therefore, a program that educates growers as to the necessity of IRM and provides guidance as to how to deploy IRM should be an integral part of any resistance management strategy. The 2000 SAP also suggested that a comprehensive education program may help increase IRM compliance (U.S. EPA 2001). Ideally, the educational messages presented to growers should be consistent (among different registrants, if applicable for CRW) and reflect the most current resistance management guidelines. Specific examples of education tools for growers can include grower guides, technical bulletins, sales materials, training sessions, internet sites, toll-free numbers for questions or further information, and educational publications.

Monsanto's grower education program for MON 863 was reviewed in U.S. EPA (2004a) and found to be acceptable. Components of the educational program include a Technology Use Guide (for growers), a Seed Resource Guide (for seed dealers), an interactive CD-ROM, on-line training module (developed in cooperation with the National Corn Growers Association (NCGA)), and educational/promotional meetings where IRM issues will be discussed.

3. MON 863 x MON 810 (YieldGard® Plus; EPA Reg. No. 524-545)

Monsanto has developed an IRM plan for YieldGard® Plus Corn. YieldGard® Plus is a stacked corn product that expresses Cry3Bb1 (MON 863) and Cry1Ab (MON 810) for control of corn rootworm and corn borers. An amendment to Monsanto's IRM plan in Volume 1 was submitted to EPA on April 1, 2003 (reviewed in U.S. EPA (2003b)). Since YieldGard® Plus expresses the Cry1Ab and Cry3Bb1 proteins, an IRM plan needs to address both European corn borer and corn rootworm. Monsanto's IRM

plan for YieldGard® Plus considers European corn borer and corn rootworm resistance management and takes a conservative approach when strategies differ between the target pests.

Refuge Requirements

Based upon growers' agronomic practices and pesticide use, growers may plant one refuge for European corn borers and corn rootworms or separate refuges for each pest. A grower that adopts the common refuge option would be required to plant a minimum of a 20% non-*Bt* structured refuge adjacent to or within YieldGard® Plus Corn fields. Refuges acres should be planted as continuous blocks adjacent to or within fields, perimeter strips, or strips within YieldGard® Plus Corn fields. Monsanto is recommending that in-field strips should be at least four row and preferably six rows wide. Agronomic practices should be comparable for YieldGard® Plus Corn and refuge acres. For example, if YieldGard® Plus Corn acres are planted continuously or as first-year corn, then the non-*Bt* refuge acres should also be planted continuously or as first-year corn, respectively. Non-*Bt* insecticides may be applied to refuge acres to control corn root larvae but may only be applied to refuge acres when corn rootworm adults are present if YieldGard® Plus Corn acres are also treated.

Growers that choose the separate refuge option must plant a distinct refuge for corn rootworm and European corn borer. A 20% non-corn rootworm-protected corn refuge must be planted to delay corn rootworm resistance to YieldGard® Plus Corn. An additional 20% non-*Bt* corn must also be planted to delay European corn borer resistance. The corn rootworm refuge must be planted with corn that does not contain the Cry3Bb1 protein. Corn that only contains the Cry1Ab protein, however, may be planted if a separate non-*Bt* corn refuge is planted to delay European corn borer resistance. The corn rootworm refuge should be planted as continuous blocks adjacent to or within fields, perimeter strips, or strips within YieldGard® Plus Corn (at least 4 rows), and utilize comparable agronomic practices as the YieldGard® Plus Corn acres. European corn borer refuges may be planted within fields as blocks or strips, adjacent to fields, or up to ½ mile from YieldGard® Plus Corn acres. Non-*Bt* insecticides may be applied to refuge acres to control corn rootworm larvae but may only be applied to refuge acres when corn rootworm adults are present if YieldGard® Plus Corn acres are also treated. Non-*Bt* insecticides may be applied to refuges to control the European corn borer, corn earworm, or southwestern corn borer if economic injury levels occur. The refuge requirements for YieldGard® Plus and stacked Cry3Bb1/Cry1Ab PIPs are summarized in Table 12.

Resistance Monitoring

Monsanto is required to monitor for pest resistance to the Cry1Ab and Cry3Bb1 proteins in YieldGard® Plus Corn. Monsanto has folded YieldGard® Plus Corn into the existing resistance monitoring plans for lepidoptera (Cry1Ab) and CRW (Cry3Bb1). A complete discussion of the resistance monitoring program for Cry1Ab and lepidopteran pests is included in the U.S. EPA (2010b). Resistance monitoring for Cry3Bb1 and CRW is described in section II(D)(2)(b)(vii) of this Biopesticides Registration Action Document (BRAD).

Remedial Action

A Remedial Action Plan is required for YieldGard® Plus Corn and must be implemented if resistance is detected. This plan is the same as the remedial action plans developed for European corn borer (detailed in U.S. EPA (2010b)) and corn rootworm (described in section II(D)(2)(b)(viii) of this BRAD).

Grower Agreements

Growers are required to sign an agreement similar to the agreements growers currently sign to plant MON 810 or MON 863 corn. This signed agreement contractually obligates growers to comply with refuge requirements.

Compliance Assurance Plan

Monsanto is required to implement a CAP that will evaluate and promote grower compliance. YieldGard® Plus Corn is included with the ABSTC compliance program for *Bt* corn (described in section II(D)(2)(b)(ix) of this BRAD).

Grower Education

Grower education programs are required for YieldGard® Plus Corn. The elements of this program are the same as for MON 863 and MON 810 corn but must describe how to deploy refuges for both lepidopteran and rootworm pests.

4. MON 88017 (YieldGard® VT RW; EPA Reg. No. 524-551)

The Cry3Bb1 protein expressed in MON 88017 corn is functionally and physiologically similar to that expressed in MON 863. The proteins differ by only 1 amino acid of 653 (99.8% homology) and are expressed at comparable levels in the plant. To test for functional equivalence, Monsanto conducted susceptibility assays with Colorado potato beetle (CPB) and WCRW, as well as field efficacy tests against CRW larvae. The susceptibility assays involved diet incorporation of Cry3Bb1 from each hybrid to determine LC_{50} values for the test insects. The results were similar for both Cry3Bb1 variants: (1) for CPB, the MON 88017 variant (Cry3Bb1.pvzmir39) had an LC_{50} of 0.84 microgram per milliliter (µg/mL), while the MON 863 variant (Cry3Bb1.11098) had an LC_{50} of 0.95 µg/mL; (2) for WCRW, the LC_{50} was 139 µg/mL for the MON 88017 variant and 100 µg/mL for the MON 863 variant. Field efficacy trials were conducted with MON 88017, MON 863, and non-expressing control plants using artificial infestations of WCRW eggs. Efficacy was measured as protection against feeding damage using a root rating scale. Seven weeks after infestation, the root damage ratings (RDR = 0.12) were identical for MON 88017 and MON 863, both of which were significantly lower than the level of damage on the control plants (RDR = 1.47).

In addition to structural and functional analysis of the Cry3Bb1 toxin, Monsanto also determined protein expression levels in MON 88017 relative to those for MON 863. Using enzyme-linked immunosorbent assay (ELISA) techniques, leaf, root, pollen, silk, grain, and stover tissues were analyzed for the amount of Cry3Bb1 protein both in dry weight and fresh weight tissues. The results showed that the protein expression in MON 88017 was comparable to MON 863: expression was slightly higher in young leaf, stover, and silk tissues; slightly lower in pollen; and the same in forage, forage root, and grain tissues. Only the expression in silk was significantly different. When tracked through the growing season, the amount of Cry3Bb1 protein declined in MON 88017 leaf, whole plant, and root tissue in a manner similar to that observed for MON 863.

The IRM plan developed for MON 863 corn is compatible with MON 88017 corn (MRID No. 461817-01; reviewed in U.S. EPA (2005b)), and all aspects of the MON 863 IRM plan are to be followed for MON 88017. A complete description of the MON 863 (Cry3Bb1) IRM plan is found in section II(D)(2) of this BRAD.

5. MON 88017 x MON 810 (YieldGard® VT Plus; EPA Reg. No. 524-552)

Monsanto's submission indicates that the Cry3Bb1 and Cry1Ab toxins expressed in MON 88017 x MON 810 are "physiologically and functionally" equivalent to that expressed in MON 863, MON 88017, and MON 810. To demonstrate the physiological equivalence, Monsanto investigated the amino acid sequences of the Cry3Bb1 toxins produced in both MON 88017 and MON 863. The Cry3Bb1 proteins produced in MON 88017 and MON 863 share an amino acid sequence identity of >99.8%, differing from one another by only 1 of 653 amino acids. Since the Cry1Ab toxin was introduced using conventional breeding with MON 810, the toxins in MON 88017 x MON 810 and MON 810 should be identical. To test for functional equivalence, field efficacy tests were conducted against CRW and ECB larvae. Four treatments were used: MON 88017 x MON 810, MON 88017, MON 810 (crossed with a glyphosate-tolerant hybrid), and a non-expressing control (a glyphosate-tolerant hybrid). For ECB, evaluations of natural infestations were used, which were supplemented by artificial infestations at the whorl stage. Damage (efficacy) was determined by assessing leaf damage (LDR) using the Modified Guthrie Scale (0 = no damage; 9 = high damage). CRW efficacy was also evaluated with artificial infestations of WCRW, which was done at the second leaf stage (V2). Damage was assessed using a RDR scale (Oelson Node Injury Scale). The results for ECB efficacy (tabulated after 21 days) showed that both MON 88017 x MON 810 and MON 810 alone had low amounts of leaf damage (LDR = 0.8 and 0.9, respectively), while the MON 88017 alone and non-expressing control had significantly higher levels of damage (LDR = 2.7 for both). For WCRW (determined after 6–7 weeks), both MON 88017 x MON 810 and MON 88017 alone had significantly greater root protection (RDR = 0.1 for both) than MON 810 alone or the non-expressing control (RDR = 1.24 and 1.35, respectively).

In addition to the structural and functional analysis of the Cry3Bb1 and Cry1Ab toxins, Monsanto also determined protein expression levels in MON 88017 x MON 810 relative to those for MON 88017 and MON 810. MON 88017 had been previously compared with MON 863 for Cry3Bb1 expression, which was found to be almost identical. Using ELISA techniques, young leaf, young root (Cry3Bb1 only), pollen (Cry3Bb1 only), forage (leaf), forage root (Cry3Bb1 only), and grain tissues were analyzed for

the amount of Cry3Bb1 and Cry1Ab protein both in dry weight and fresh weight tissues. The results showed that the Cry3Bb1 protein expression in MON 88017 x MON 810 was comparable to MON 88017 in all tissues. Expression in MON 88017 x MON 810 was slightly lower in young root and grain tissues and was higher in all other tested tissues, though none of the differences were statistically significant. For Cry1Ab, expression in MON 88017 x MON 810 was also comparable to MON 810, with only slight insignificant differences in young leaf, forage leaf, and grain tissues.

The IRM plan developed for YieldGard® Plus (MON 863 x MON 810), consisting of CRW (MON 863) and Lepidoptera (MON 810) components, is compatible with MON 88017 x MON 810 corn (MRID No. 461850-01; reviewed in U.S. EPA (2005c)), and all aspects of the YieldGard® Plus IRM plan are to be applied to MON 88017 x MON 810. The refuge requirements for MON 88017 x MON 810 corn are summarized in Table 12.

Table 12. Comparison of Event MON 810 (Lepidopteran) and MON 863/88017 (Rootworm) IRM Requirements for Monsanto's Stacked *Bt* Corn PIPs (YieldGard® Plus and YieldGard® VT Plus Corn).

Requirements	MON 810 (Lepidopteran Refuge)	MON 863/88017 (Rootworm Refuge)	YieldGard® Plus/VT Plus Corn (Combined Refuge)
Refuge Size	≥20%	≥20%	≥20%
Refuge Placement	≤½ mile	Adjacent or within field	Adjacent or within field
Refuge Configuration	Block, in-field strips (≥4 rows), or edges	Block or strips (≥4 rows)	Block or strips (≥4 rows)
Refuge Management	Any corn rotation meeting placement & configuration requirements. Insecticides can be used in refuge to control ECB & southwestern corn borer when above economic thresholds. Microbial *Bt* insecticides are not allowed.	Same corn rotation as *YGRW* (e.g., first-year corn or corn followed by corn). Conventional insecticides or seed treatments can be used in the refuge to control CRW larvae & other soil pests. If the refuge is treated with a foliar insecticide labeled for CRW control when CRW adults are present, then *YGRW* also must be treated. (Not applicable)	Same corn rotation as *YG Plus* (e.g., first-year corn or corn followed by corn). Conventional insecticides or seed treatments can be used in the refuge to control CRW larvae & other soil pests. If the refuge is treated with a foliar insecticide labeled for CRW control when CRW adults are present, then *YG Plus* also must be treated. Microbial *Bt* insecticides are not allowed.
Refuge Corn Types	Conventional (Non-*Bt*)	Conventional (Non-*Bt*) *YGCB* (a corn borer refuge planted ≤½ mile also will be required) *Roundup Ready corn (Non-Bt)*	Conventional (Non-*Bt*) *YGCB* (an additional refuge for corn borers will be required) *Roundup Ready corn (Non-Bt)*

YGCB = YieldGard® Corn Borer; *Bt* corn with lepidopteran corn borer protection only (i.e., MON 810).
YGRW = YieldGard® Rootworm; *Bt* corn with corn rootworm protection only (i.e., MON 863).

6. Conclusions

A 20% non-*Bt* corn refuge for Cry3Bb1 corn has been employed since the registration of MON 863 in 2003. As a condition of the MON 863 registration, Monsanto was required to submit data on corn rootworm biology, ecology, susceptibility to Cry3Bb1, and genetics of potential resistance. These data, as well as other published literature, support the adequacy of the current IRM plan for MON 863, MON

863 x MON 810, MON 88017, and MON 88017 x MON 810. Since there are still uncertainties associated with potential rootworm resistance to the Cry3Bb1 toxins, however, the adequacy of the IRM strategy should be periodically reevaluated.

The non-*Bt* corn refuge should be planted as continuous blocks adjacent to the MON 863 fields, as perimeter strips, or as non-transgenic strips planted within the transgenic field. A 20% non-*Bt* corn refuge is necessary to produce an adequate number of CRW susceptible to the Cry3Bb1 protein. Considering the limited movement of CRW larvae, planting refuges close to transgenic fields in large blocks is preferred to narrow strips (Gray 1999; Meinke *et al*. 2001). If a 20% refuge is planted as row strips within a corn field, then it should be planted as at least four or more consecutive rows (Hibbard *et al*. 2003).

Seed and granular insecticide treatments to control CRW larvae are acceptable on refuge acres. It is not acceptable, however, to treat refuges for adult CRW control as these treatments may diminish the effectiveness of the refuge. If growers spray their corn fields with insecticides to control pests other than CRW, then all acres (*Bt* and non-*Bt*) should be treated identically. *Bt* fields and the non-*Bt* refuge acres should be treated with identical agronomic practices, such as irrigating all corn (*Bt* and non-*Bt*) at the same time. To ensure the production of similar numbers of CRW, *Bt* and non-*Bt* corn should be planted in fields with similar backgrounds. For example, if MON 863 hybrids are planted on continuous corn fields, then the non-*Bt* refuge should be planted on continuous corn fields or both should be planted on first-year corn acres. Likewise, non-*Bt* refuges should be planted on first-year corn fields if the MON 863 hybrids are planted on first-year corn fields.

7. IRM Terms and Conditions of Registration

The terms and conditions for each of the Cry3Bb1 registrations contain a complete description of the IRM requirements. Details are provided on the requirements for refuge (size and structure), resistance monitoring, remedial action, compliance assurance, grower education, and annual IRM reports. For more information, please refer to the document, in Docket Number EPA-HQ-OPP-2010-0607, presenting the registration terms and conditions established with the 2010 amendments.

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E. Benefits and Public Interest Findings for Initial Registrations of Corn Event MON 863 and MON 88017

1. Background

Corn is the largest cultivated crop grown in the U.S. in terms of acreage planted and net value. The crop was planted on 79.5 million acres in the year 2000, yielding 10 billion bushels with a net value of $18.4 billion. There are nearly 410 million acres of agricultural land being used to grow crops nationwide, including Conservation Reserve Program lands. Corn rootworm (CRW, *Diabrotica* spp.) is one of a spectrum of insect pests that a farmer may choose to control. Other insect pests include cutworms, wireworms, white grubs, flea beetles, seedcorn maggots, black cutworms, and corn borer species. CRW, however, is one of the most damaging insect pests of corn and is responsible for economic damages (costs associated with insecticides and crop losses) totaling nearly $1 billion (Gray 2000). The three CRW control methods have been (1) use of crop rotation, (2) soil-applied insecticides, and (3) limited use of rescue-treatments for CRW adult beetles.

MON 863 and MON 88017 corn rootworm-protected corn both contain the *cry*3Bb1 gene that produces the insecticidal crystal protein, Cry3Bb1. MON 863 also has a *nptII* marker gene that encodes neomycin phosphotransferase II, while MON 88017 has a *cp4 epsps* gene that encodes CP4 5-enolpyruvylshikimate-3-phosphate synthase, conferring resistance to glyphosate. Cry3Bb1 corn is targeted against the CRW complex, comprised primarily of the northern corn rootworm (NCRW, *Diabrotica barberi* Smith and Lawrence), western corn rootworm (WCRW, *Diabrotica virgifera virgifera* LeConte*)*, and Mexican corn rootworm (MCRW, *Diabrotica virgifera zeae* Krysan and Smith). One additional *Diabrotica* species, the southern corn rootworm (SCRW, *Diabrotica undecimpunctata howardi* Barber) is considered a relatively minor pest of corn that inhabits the southeastern coastal regions of the U.S. CRW accounts for more chemical pesticide usage on corn than does any other pest; approximately 28 million acres of corn are infested with CRW. In the year 2000, approximately 8 million pounds of insecticidal active ingredient, costing $172 million, were applied to 14 million acres of corn to reduce CRW damage. There were approximately 24 million acres of corn treated with insecticides for CRW and other pests (e.g., grubs, maggots, cutworms, wireworms). The National Agricultural Statistics Service's figures from 2001 indicate that 9.8 million pounds of insecticide active ingredients, specifically registered for CRW control, were applied on more than 31% of the planted acres. Left untreated, CRW can cause severe yield loss, typically in the range from 8% to 16%, although reductions in yield may be as high as 28%.

2. 2003 Corn Event MON 863 Public Interest Finding (Reviewed in U.S. EPA (2003a and 2003b))

The criteria for determining whether registration of a pesticide chemical is in the public interest are set forth in a Federal Register Notice dated March 5, 1986 (51 Federal Register (FR) 7628). There is a presumption that registration of a pesticide chemical is in the public interest if one of the following criteria is met: (1) the use is for a minor crop; (2) the use is a replacement for another pesticide that is of continuing concern to the Agency; (3) the use is one for which an emergency exemption under section 18 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) has been granted (i.e., the basis

for the exemption was lack of a registered alternative product); or (4) the use is against a pest of public health significance. Further, the Environmental Protection Agency (EPA) may determine that such a registration is in the public interest on the basis of the following criteria: (1) there is a need for the new chemical that is not being met by currently registered pesticides; (2) the new pesticide is comparatively less risky to health or the environment than currently registered pesticides; or (3) the benefits (including economic benefits) from the use of the new active ingredient exceed those of alternative registered pesticides and other available non-chemical techniques.

EPA determines that conditional registration of MON 863 is in the public interest given conventional chemicals, currently used for CRW control, that are of continuing concern to the Agency as indicated by the following factors:

1. **Special review**. Certain pesticides that are used for CRW control have been reviewed under EPA Special Review (40 Code of Federal Regulations (CFR) Part 154) because the use of these pesticides may result in unreasonable adverse effects to humans or the environment. The pesticides in this category are dimethoate, phorate, and terbufos; terbufos and phorate are being evaluated for reregistration.

2. **Acute avian risk from granular pesticides**. In 1992, EPA issued an analysis that indentified 14 granular pesticides believed to pose potentially higher risk of killing birds due to their acute toxicity and availability in the environment (U.S. EPA 1992). Among these granular pesticides are several that are still used for CRW control: carbofuran (no longer used as a granular but still a pesticide of Agency concern), phorate, terbufos, and chlorpyrifos. The results of the Agency's initiative to reduce exposure to these highly toxic granular pesticides are presented in the 1994 EPA report, "Avian Granular Risk Reduction Initiative."

3. **Restricted use**. Many of the granular pesticides used for CRW control have been classified as restricted use (due to adverse environmental effects under use practices), limiting the use of these chemicals to certified pesticide applicators. Pesticides classified as restricted use include chloroethoxyfos, phorate, terbufos, tefluthrin, and the commercial combination of tebupirimfos and cyfluthrin (i.e., Aztec®).

4. **Food Quality Protection Act of 1996**. EPA must reassess all existing tolerances to be sure that they meet the standard of "reasonable certainty of no harm." The EPA is required to first consider those pesticides that pose the highest risk to humans. EPA is reviewing the organophosphate and carbamate pesticides because of their known risk of acute and chronic toxicity to humans and wildlife. The organophosphates and carbamates share the same mode of action. The organophosphate insecticides used for CRW control include chlorpyrifos, terbufos, phorate, chloroethoxyfos, dimethoate, and tebupirimphos, while a carbamate insecticide used for CRW control is carbofuran.

In addition, EPA also determines, in accordance with the criteria set forth in the Federal Register Notice dated March 5, 1986, that MON 863 qualifies for a positive public interest finding. To qualify for a positive public interest finding, the product must demonstrate advantages in terms of the need for the chemical and its comparative benefits, risks, and costs. Monsanto Company ("Monsanto") has submitted two public interest documents and other supporting documents that present the potential benefits of MON 863 (Master Record Identification Numbers (MRID Nos.) 450297-01, 456530-01, 456530-02, 453613-03, 455382-08, 456923-01, 455770-01). EPA has reviewed the submitted documents, public comments, syndicated marketing research studies, and published information. The potential benefits have been identified and evaluated

The major proposed benefits of MON 863 corn for CRW control are as follows:

- Safer for handlers, applicators, growers, and the public than current chemical alternatives
- Safer for the environment than use of available chemical pesticides
- Easier and less time consuming for farmers to use than current control options
- Comparable or improved efficacy relative to the current chemical alternatives
- Yield benefits
- Reduced use of current higher risk chemical alternatives
- Economic benefits to farmers from increased yields and decreased cost of rootworm control as compared with conventional control

The use of MON 863 CRW-protected corn is presumed to be in the public interest because it will replace or reduce the use of a number of higher risk pesticides for CRW control that are of Agency concern as discussed previously (e.g., terbufos, chlorpyrifos, and phorate). Additionally, MON 863 also has clearly identified benefits. Therefore, EPA concludes that the use of MON 863 CRW-protected corn is in the public interest and supports the conditional registration of MON 863 under FIFRA section 3(c)(7)(C).

a. Characterization and Use of Chemical Insecticides to Control Corn Rootworm

Three CRW control methods have been used for decades: (1) crop rotation (typically with soybeans), (2) soil-applied insecticides to control larvae (approximately 90% of the total CRW-treated acres), and (3) use of adulticides to control CRW adult beetles (approximately 10% of the total CRW-treated acres)(Levine and Oloumi-Sadeghi 1991). Greater than 90% of the growers use soil-applied insecticides, applied at planting to control larvae, due to greater efficacy and ease of application. Historically, crop rotation has been the primary method of controlling CRW (Levine and Oloumi-Sadeghi 1991). Crop rotation, however, is now far less effective because of the existence of a WCRW soybean rotational variant, primarily in Eastern Illinois and Western Indiana, that colonizes soybeans (Levine and Oloumi-Sadeghi 1991; Levine *et al*. 1992a) and a NCRW extended diapause (2 year extended) variant, primarily in parts of Minnesota, Iowa, and South Dakota (Krysan *et al*. 1986; Levine *et al*. 1992b). In addition, CRW has developed resistance to methyl parathion and carbaryl, both adulticides used in rescue treatments (Meinke *et al*. 1998). Therefore, growers have become increasingly dependent on chemical pesticides to limit CRW losses. EPA has registered 36 insecticide products for control of CRW (see

Table 1). The insecticides used to control corn rootworm in conventionally grown (non-*Bt*) corn consist mainly of organophosphates (9), carbamates (3), synthetic pyrethroids (6), and phenyl pyrazole (1) classes of chemistry. Twenty-five products are classified as "restricted use." All 36 insecticides are toxic to extremely toxic to fish, aquatic invertebrates, bees, and/or wildlife. Three products have been involved in the Agency's Special Review process: dimethoate, phorate, and terbufos. Twenty-six of these products contain active ingredients either from the organophosphate or carbamate classes, both of which are considered to be top priorities under the Food Quality Protection Act (FQPA) and tolerance reassessment. Terbufos, phorate, chlorpyrifos, diazinon, ethoprop, carbofuran, and methomyl have presented varying levels of concern regarding avian, aquatic, and mammalian risk. EPA has specified specific risk mitigation measures for all of these chemicals and, in many cases, certain uses have been eliminated because of either human or environmental risk concerns.

Table 1. Insecticide End-Use Products Registered by EPA for Use on Corn for Control of Corn Rootworm Species (Reprinted from Pages 18-21 of MRID No. 456530-01 and Verified by EPA).

Product	Active Ingredients	Type[a]	Use Rate[b]	Use	Classification[c]
Ambush® Insecticide – Syngenta	permethrin – 25.6%	SP	0.2 lb/acre	Adult control	**WARNING. Restricted Use**; extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Asana–XL Insecticide 0.66 Emulsifiable Concentrate – DuPont	esfenvalerate – 8.4%	SP	0.05 lb/acre	Adult control	**WARNING. Restricted Use**; extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Aztec–2.1% Granular Insecticide – Bayer	tebupirimfos – 2.0% cyfluthrin – 0.1%	OP SP	0.15 lb/acre 0.01 lb/acre	Larval control	**WARNING. Restricted Use**; toxic to fish and wildlife
Baythroid–2 Emulsifiable Pyrethroid Insecticide – Bayer	cyfluthrin – 25%	SP	0.04 lb/acre	Adult control	**DANGER. Restricted Use**; extremely toxic to fish and aquatic invertebrates, highly toxic to bees, may cause allergic skin reactions
Capture–2EC Insecticide/Miticide – FMC	bifenthrin – 25.1%	SP	0.3 lb/acre	Larval control	**WARNING. Restricted Use**; extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Chlorfos–15G Insecticide Granular – Griffin LLC	chlorpyrifos – 15%	OP	2.02 lb/acre	Larval control	**CAUTION.** Toxic to birds and wildlife, extremely toxic to fish and aquatic organisms
Chlorfos–4E Insecticide – Griffin LLC	chlorpyrifos – 42%	OP	2.52 lb/acre	Adult & Larval control	**WARNING.** Toxic to birds and wildlife, extremely toxic to fish and aquatic organisms
Counter–CR Systemic Insecticide-Nematicide – American Cyanamid Company	terbufos – 20%	OP	1.30 lb/acre	Larval control	**DANGER. Restricted Use**; fatal if swallowed, inhaled, or absorbed through skin, extremely toxic to fish and wildlife

Product	Active Ingredients	Type[a]	Use Rate[b]	Use	Classification[c]
D-z-n–Diazinon AG500 Insecticide – Syngenta	diazinon – 48%	OP	0.48 lb/acre	Adult control	**CAUTION. Restricted Use**; highly toxic to birds, fish, and other wildlife, highly toxic to bees
D-z-n–Diazinon AG600 WBC Insecticide – Syngenta	diazinon – 56%	OP	0.45 lb/acre	Adult control	**CAUTION. Restricted Use**; highly toxic to birds, fish, and other wildlife, highly toxic to bees
Declare–Emulsifiable Insecticide Concentrate – Griffin LLC	methyl parathion – 45.11%	OP	0.22 lb/acre	Adult control	**DANGER. Restricted Use**; fatal if swallowed, inhaled, or absorbed through skin, highly toxic to aquatic invertebrates and wildlife, highly toxic to bees
Diazinon 500-AG Organophosphate Insecticide – UAP	diazinon – 48%	OP	0.48 lb/acre	Adult control	**CAUTION. Restricted Use**; highly toxic to birds, fish, and other wildlife, highly toxic to bees
Dimethoate 4 EC Systemic Insecticide – Helena	dimethoate – 44.8%	OP	0.45 lb/acre	Adult control	**WARNING.** Toxic to wildlife and aquatic invertebrates, highly toxic to bees
Dimethoate 400 Systemic Insecticide-Miticide – UAP	dimethoate – 43.5%	OP	0.44 lb/acre	Adult control	**WARNING.** Toxic to wildlife and aquatic invertebrates, highly toxic to bees
5 lb Dimethoate Systemic Insecticide – Helena	dimethoate – 57%	OP	0.46 lb/acre	Adult control	**DANGER.** Toxic to wildlife and aquatic invertebrates, highly toxic to bees
Force–3G Insecticide – Syngenta	tefluthrin – 3%	SP	0.17 lb/acre	Larval control	**CAUTION. Restricted Use**; very highly toxic to freshwater and estuarine fish and invertebrates
Fortress–2.5G Granular Insecticide – DuPont	chlorethoxyfos – 2.5%	OP	0.16 lb/acre	Larval control	**DANGER. Restricted Use**; toxic to wild mammals, birds, fish, and aquatic invertebrates

Product	Active Ingredients	Type[a]	Use Rate[b]	Use	Classification[c]
Fortress 5G Granular Insecticide – DuPont	chlorethoxyfos – 5%	OP	0.16 lb/acre	Larval control	**DANGER. Restricted Use**; toxic to wild mammals, birds, fish, and aquatic invertebrates
Furadan 4F Insecticide/ Nematicide – FMC	carbofuran – 44%	C	0.88 lb/acre	Adult & larval control	**DANGER. Restricted Use**; poisonous if swallowed or inhaled, toxic to fish, birds, and other wildlife, highly toxic to bees, can seep or leach through soil and can contaminate groundwater
Lannate LV Insecticide – DuPont	methomyl – 29%	C	0.65 lb/acre	Adult control	**DANGER. Restricted Use**; fatal if swallowed, toxic to fish, aquatic invertebrates, and mammals, highly toxic to bees, known to leach through soil into groundwater
Lannate SP Insecticide – DuPont	methomyl – 90%	C	0.45 lb/acre	Adult control	**DANGER. Restricted Use**; fatal if swallowed, may cause blindness, toxic to fish, aquatic invertebrates, and mammals, highly toxic to bees, known to leach through soil into groundwater
Lorsban 15G Granular Insecticide – Dow AgroSciences	chlorpyrifos – 15%	OP	2.03 lb/acre	Larval control	**CAUTION.** Toxic to birds and wildlife, extremely toxic to fish and aquatic organisms
Lorsban-4E Insecticide – Dow AgroSciences	chlorpyrifos – 44.9%	OP	2.69 lb/acre	Adult & larval control	**WARNING.** Toxic to birds and wildlife, extremely toxic to fish and aquatic organisms
Mocap *10% Granular Nematicide Insecticide* – Aventis CropScience	ethoprop – 10%	OP	3.53 lb/acre	Larval control	**WARNING.** Toxic to aquatic organisms and wildlife

Product	Active Ingredients	Type[a]	Use Rate[b]	Use	Classification[c]
Mocap–*EC Nematicide-Insecticide* – Aventis CropScience	ethoprop – 69.6%	OP	3.34 lb/acre	Larval control	**DANGER. Restricted Use**; toxic to aquatic organisms and extremely toxic to birds
Penncap-M–Microencapsulated Insecticide – Elf Atochem	methyl parathion – 22%	OP	0.44 lb/acre	Adult control	**WARNING. Restricted Use**; highly toxic to aquatic invertebrates and wildlife
Phorate 20 G Organophosphate Insecticide – UAP	phorate – 20%	OP	1.3 lb/acre	Adult & larval control	**DANGER. Restricted Use**; extremely toxic to fish and wildlife
Pounce® WSB Insecticide – FMC Corporation	permethrin – 24.7%	SP	0.2 lb/acre	Adult control	**WARNING. Restricted Use**; extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Pounce® 3.2 EC Insecticide – FMC Corporation	permethrin – 38.4%	SP	0.2 lb/acre	Adult control	**CAUTION. Restricted Use**; extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Pounce® 25 WP Insecticide – FMC Corporation	permethrin – 25%	SP	0.2 lb/acre	Adult control	**WARNING. Restricted Use**; extremely toxic to fish and aquatic invertebrates, highly toxic to bees
Regent–4 SC Insecticide – Aventis CropScience	fipronil – 39.4%	PP	0.13 lb/acre	Larval control	**WARNING. Restricted Use**; toxic to birds, fish and aquatic invertebrates
Sevin–Brand 80S Carbaryl Insecticide – Aventis CropScience	carbaryl – 80%	C	2.0 lb/acre	Adult control	**WARNING.** Extremely toxic to aquatic and estuarine invertebrates, highly toxic to bees
Sevin–Brand XLR PLUS Carbaryl Insecticide – Aventis CropScience	carbaryl – 44.1%	C	1.76 lb/acre	Adult control	**CAUTION.** Extremely toxic to aquatic and estuarine invertebrates, highly toxic to bees

Product	Active Ingredients	Type[a]	Use Rate[b]	Use	Classification[c]
Thimet 20-G Soil and Systemic Insecticide – American Cyanamid	phorate – 20%	OP	1.3 lb/acre	Larval control	**DANGER. Restricted Use**; extremely toxic to fish and wildlife
Thimet 20-G Soil and Systemic Insecticide – American Cyanamid	phorate – 20%	OP	1.3 lb/acre	Larval control	**DANGER. Restricted Use**; extremely toxic to fish and wildlife
Warrior-Insecticide with Zeon Technology – Syngenta	lambda-cyhalothrin – 11.4%	SP	0.03 lb/acre	Adult control	**WARNING. Restricted Use**; extremely toxic to fish and aquatic organisms and toxic to wildlife, highly toxic to bees

a – OP: organophosphate; SP: synthetic pyrethroid; C: carbamate; PP: phenyl pyrazole
b – maximum labeled use rate expressed in pounds of active ingredient per acre (assume that 1 liquid pt = 1 pound)
c – precautionary language as stated on label

In the year 2000, approximately 8 million pounds of insecticidal active ingredient, costing $172 million, were applied to 14 million acres of corn to reduce CRW damage (see Table 2 below). These data were independently verified by EPA. There were approximately 24 million acres of corn treated with insecticides; growers indicated that CRW was the sole target pest on approximately 7 million acres and one of a number of pests on the remaining treated acres. CRW-targeted acres (14 million) that received an insecticide treatment represented 18% of the total acres of corn planted in the continental U.S. and 59% of the total acres receiving any insecticide treatment in 2000. Continuous corn and first-year corn acres (rotated acres) received 58% and 42% of the CRW-targeted insecticide applications, respectively. Continuous corn use areas include western Iowa, Nebraska, eastern Colorado, eastern South Dakota, the panhandle areas of Texas and Oklahoma, northeastern New Mexico, and southern Minnesota. Rotational acres are located predominantly in eastern Iowa, most of Minnesota, Wisconsin, Missouri, Illinois, Indiana, Michigan and other areas of the Eastern Corn Belt.

The CRW treatments on 14 million acres of corn do not include acreage where the expected level of infestation is below the economic threshold (i.e., where the expected loss is less than the $15.00 cost of treatment). National estimates of infested acreage are not published (unlike the Cotton Council, for example, that publishes estimates of infested acreage). Market analyses estimate all infested acreage with CRW to be around 28 million acres. This estimate is reasonable with what could be expected on the basis of yield losses without treatment and prices in year 2000. At depressed corn prices of $2 per bushel and yields at 175 bushels/acre, a maximum yield loss of 8.5% would result in a $30 loss. Half of the infested acres would be below the $15.00 cost of treatment. If corn prices rise or the cost of control decreases, the percent of infested acres that is treated would likely increase.

The infested acreage is expected to grow by 2.6% per year, implying that the 28 million infested acres in year 2000 will grow to 39 million infested acres by the year 2013. These factors need to be considered when projecting the amount of chemical acre treatments and adopted acres of MON 863.

Table 2. Insecticide Usage on Corn in Year 2000 (Reprinted from Page 22 of MRID No. 456530-01).

Parameter	Continuous Corn	First-Year Corn	All Corn
Acres planted (x1,000)	22,269	57,310	79,579
Total insecticide-treated acres (x1,000)	11,590	12,518	24,108
Total insecticide active ingredient applied (lb x1,000)	6,332	6,011	12,343
CRW-targeted acres (x1,000)	**8,271**	**5,926**	**14,197**
Active ingredient applied to CRW-targeted acres (lb x1,000)	4,699	3,137	7,836
Average active ingredient rate applied (lb/ac)	0.568	0.529	0.552
Average cost per acre	$11.95	$12.27	$12.08
Total cost of CRW insecticide purchased (x1,000)	$98,811	$72,699	$171,510

Only a few of the 36 products listed in Table 1, registered for control of CRW, dominate the market. Table 3 below shows that five active ingredients (tefluthrin, chlorpyrifos, terbufos, fipronil, and cyfluthrin/tebupirimfos) are applied to 86% of the acres treated. Three of these active ingredients are organophosphates and three are classified as restricted use. In terms of pounds of insecticide applied, chlorpyrifos and terbufos (both organophosphates), account for 77% of the total insecticide products (almost 6 million pounds) applied to CRW-targeted acres. These 11 active ingredients (in Table 3) accounted for 98.3% of the total quantity of insecticide applied to CRW-targeted acres in 2000.

Table 3. Insecticide Active Ingredients Applied to Corn Rootworm-Targeted Acres in Year 2000 (Reprinted from page 24 of MRID No. 456530-01).

Active Ingredient	Acres Treated (x1,000)	Pounds Applied (Formulated Product)
Carbofuran	342	242,379
Chlorethoxyfos	361	55,485
Chlorpyrifos	**3,557**	**3,765,310**
Cyfluthrin/Tebupirimfos	1,326	179,527
Fipronil	1,498	158,141
Lambda-cyhalothrin	179	3,846
Methyl parathion	367	142,011
Permethrin	246	24,344
Phorate	508	588,380
Tefluthrin	3,570	400,339
Terbufos	2,044	2,146,761
Total	**13,998**	**7,706,523**

Table 4 provides a listing of the 13 major end-use products that are applied to CRW-targeted areas (Table 1 lists end-use products that are labeled for CRW control). The use of five products—Aztec 2.1% Granular Insecticide, Counter CR Systemic Insecticide-Nematicide, Force 3G Insecticide, Lorsban 15G Granular Insecticide, and Regent 4 SC Insecticide—accounted for applications to 83% of the CRW-targeted areas. These products are all for larvicide rather than adulticide use. All of these products are restricted use, except Lorsban 15G Granular Insecticide. A small number of seed-applied insecticides

have been recently approved for use: Gaucho seed-applied insecticide (Gustafson, LLC), Prescribe seed-applied insecticide (Gustafson, LLC), and Force ST seed-applied insecticide (Syngenta). Imidacloprid is the active ingredient in Gaucho and Prescribe, and tefluthrin is the active ingredient in Force ST. MRID No. 456530-01 describes that the performance of these seed-applied insecticides is inconsistent and weak under conditions of high CRW pressure and, further, that these products do not perform as well as most soil-applied insecticides.

Table 4. Insecticide End-Use Products Used for Control of Corn Rootworm in Year 2000 (Reprinted from page 25 of MRID No. 456530-01)

Product[a]	Average Cost ($/A)	Adult (A) Control Larval (L) Control	EPA Classification	Acres Treated (x1,000)
Aztec 2.1% Granular Insecticide (tebupirimfos/cyfluthrin)	$13.05	L	Restricted	1,327
Counter CR Systemic Insecticide-Nematicide (terbufos)	$13.10–$13.50	L	Restricted	2,044
Force 3G Insecticide (tefluthrin)	$14.48	L	Restricted	3,570
Fortress 5G Granular Insecticide (chlorethoxyfor)	$14.65	L	Restricted	361
Furadan 4F Insecticide/ Nematicide (carbofuran)	$11.74	L	Restricted	342
Lorsban 15G Granular Insecticide (chlorpyrifos)	$11.79	L	Unrestricted	3,165
Lorsban 4E Insecticide (chlorpyrifos)	$10.52	A	Unrestricted	374
Penncap-M Microencapsulated Insecticide (methyl parathion)	$6.79	A	Restricted	330
Pounce 3.2 EC Insecticide (permethrin)	$4.42	A	Restricted	224

Product[a]	Average Cost ($/A)	Adult (A) Control Larval (L) Control	EPA Classification	Acres Treated (x1,000)
Regent 4 SC Insecticide (fipronil)	$14.65	L	Restricted	1,392
Regent 80 WG Insecticide (fipronil)	$8.57	L	Restricted	106
Thimet 20-G Soil and Systemic Insecticide (phorate)	$10.90–$12.74	L	Restricted	508
Warrior Insecticide with Zeon Technology (lambda-cyhalothrin)	$7.37	A	Restricted	173
Total				
Adult Control	$4.42–$10.52			8%
Larval Control	$8.57–$14.65			92%
Restricted Use				73%
Unrestricted				27%

a – active ingredient stated in parentheses

b. Comparative Toxicity to Humans (MRID Nos. 456530-01 and 450297-01)

MON 863 CRW-protected corn is safer for handlers, applicators, farmers, and the public than chemical pesticides in current use. Adoption of MON 863 corn hybrids will reduce the occupational, farmer, and public risks associated with the manufacture, transportation, storage, handling, application, and disposal of conventional insecticides. Many comments were received concerned with the potential contact to growers, their families, and communities with the application, drift, and on-farm storage of toxic materials. At product maturity, MON 863 hybrids have the potential to reduce insecticide applications by millions of pounds. This reduction of insecticide use will lead to both reduced human and environmental risks. The potential insecticide use reduction caused by adoption of MON 863 corn hybrids is discussed in section II(E)(2)(g) of this Biopesticides Registration Action Document (BRAD).

Virtually all of the registered conventional insecticides used to control CRW are of special concern to EPA because of risks to humans (see Table 1 and previous discussion in section II(E)(2) of this BRAD). Of the 36 insecticides registered for CRW control and listed in Table 1, 25 are classified as "Restricted Use" and 12 have the "Danger" label classification. These include products formulated with the following active ingredients: chloroethoxyfos, phorate, terbufos, tefluthrin, and the commercial combination of tebupirimfos and cyfluthrin (i.e., Aztec®). Each year there are confirmed reports of human illness associated with the registered chemical insecticide alternatives. Several of the current CRW insecticides are in Agency Special Review (i.e., dimethoate, phorate, and terbufos). Twenty-six of the 36 products contain either organophosphate or carbamate active ingredients, which are listed as top priorities for tolerance reassessment under FQPA because of their high risk to humans and the environment. Because of EPA's concern with the conventional insecticide alternatives for CRW control, special precautions are required during all stages of their life cycle, including manufacture, transportation, storage, use, and disposal.

By contrast, MON 863 corn presents minimal or no risks to humans during any stage of its life cycle, from production to ingestion to disposal. Unlike the conventional insecticide alternatives that require a tolerance (maximum allowable level of pesticide residue in food), Cry3Bb1 protein and the genetic material necessary for its production in corn has been exempted from the requirement of a tolerance given the conclusions set forth in the human health risk assessment (see section II(B) of this BRAD for additional details). Lastly, use of this new pesticide could potentially reduce use of CRW chemical pesticides by millions of pounds per year.

c. Comparative Toxicity and Potential for Adverse Environmental Effects (MRID Nos. 456530-01 and 450297-01)

All of the major chemicals used for CRW control can cause major adverse environmental effects under conditions of normal use (see Table 1 and previous discussion in section II(E)(2) of this BRAD). These products are formulated with the following active ingredients: chlorethoxyfos, phorate, terbufos, tefluthrin, methyl parathion, carbofuran, fipronil, bifenthrin, cyfluthrin, esfenvalerate, permethrin, diazinon, chlorpyrifos, dimethoate, methomyl, ethoprop, carbaryl, lambda-cyhalothrin, and the commercial combination of tebupirimfos and cyfluthrin (i.e., Aztec®). Fifteen products are labeled as "toxic," 6 as "highly toxic," 1 as "very highly toxic," and 14 as "extremely toxic" to birds, fish, and other wildlife. Each year there are confirmed reports of fish and bird poisonings associated with the registered chemical insecticide alternatives. Environmental effects from CRW chemical pesticides include toxicity and mortality in fish, birds, terrestrial mammals, aquatic invertebrates, and non-target insects. These chemicals can also spread via spray drift and runoff, thus contaminating both land and water bodies and impacting non-target organisms. Of the 36 insecticides registered for CRW control listed in Table 1, 25 are classified as "Restricted Use" and 12 have the "Danger" label classification. Table 5 compared the ecological risk for selected endpoints for the top three CRW insecticides: terbufos, chlorpyrifos, and tefluthrin. Together these three insecticides account for 63% of the acres treated (see Table 4). Tefluthrin poses lower risk than either chlorpyrifos or terbufos (see Table 5).

Table 5. Comparison of Ecological Risks Associated with Terbufos, Chlorpyrifos, and Tefluthrin.

Endpoint	Terbufos[a]	Chlorpyrifos[a]	Tefluthrin[a]
Mammalian Acute RQ	50	1	0.008
Avian Acute RQ	0.27	0.55	0.0001
Fish acute RQ	11	2	0.77
Freshwater invertebrates RQ	50	20	0.77
Marine/Estuarine Invertebrates RQ	53	162	0.87

[a] Risk is defined as the risk quotient (RQ) >level of concern (LOC).
RQ = Toxicity/Exposure. LOC = 1

Potential adverse effects on non-target organisms, resulting from the exposure to Cry3Bb1 protein, have been evaluated in a series of studies with representative avian, aquatic, and terrestrial beneficial invertebrate species as discussed in section II(C) of this BRAD.

Table 6. Summary of Results from Ecological Effects Tests with the Cry3Bb1 Proteins (Reprinted from page 35 of MRID No. 456530-01).

Test Organism	Test Substance	Results[a]	Conclusions[b]	Reference
Cladoceran (*Daphnia magna*)	Pollen	NOEC ≥2.26 µg/L	NOEC ≥141x surface water MEEC	MRID No. 449043-18
Collembola (*Folsomia candida*)	Leaf	NOEC ≥872.5 µg/g	NOEC ≥66x soil MEEC	MRID No. 449043-17
Channel Catfish (*Ictalurus punctatus*)	Grain	No effect on growth or survival at 35% of diet	No significant risk	MRID No. 449043-19
Bobwhite Quail (*Colinus virginianus*)	Grain	No effect on growth or survival at 10% of diet	No significant risk	MRID No. 449043-15
Adult Honey Bee (*Apis mellifera*)	Purified protein	NOEC ≥360 µg/mL	NOEC ≥3.8x maximum pollen level	MRID No. 449043-11
Larval Honey Bee (*Apis mellifera*)	Purified protein	NOEC ≥1,790 µg/mL as a single dose	NOEC ≥19x maximum pollen level	MRID No. 449043-10
Adult Ladybird Beetle (*Hippodamia convergens*)	Purified protein	NOEC ≥8,000 µg/g	NOEC ≥86x maximum pollen level	MRID No. 449043-14

Test Organism	Test Substance	Results[a]	Conclusions[b]	Reference
Adult Ladybird Beetle (*Hippodamia convergens*)	Pollen	No effect on growth or behavior at 50% of diet	No significant risk	MRID No. 453613-02
Larval Ladybird Beetle (*Coleomegilla maculata*)	Pollen	No effect on growth or survival at 50% of diet	No significant risk	MRID No. 455382-04
Adult Ladybird Beetle (*Coleomegilla maculata*)	Pollen	No effect on survival at 50% of diet	No significant risk	MRID No. 453613-01
Monarch Butterfly Larvae (*Danaus plexippus*)	Pollen	No effect on growth or survival	No significant risk	MRID No. 455382-05
Green Lacewing Larvae (*Chrysoperla carnea*)	Purified protein	NOEC ≥8000 µg/g	NOEC ≥86x maximum pollen level	MRID No. 449043-12
Parasitic Hymenoptera (*Nasonia vitripennis*)	Purified protein	NOEC = 400 µg/mL	NOEC ≥4.3x maximum pollen level	MRID No. 449043-13
Earthworm (*Eisenia fetifa*)	Purified protein	NOEC = 57 mg/kg	NOEC ≥4.3x MEEC in soil	MRID No. 449043-16

[a] NOEC – No Observable Effect Concentration
[b] MEEC – Maximum Expected Environmental Concentration

Results of the environmental fate studies indicate that Cry3Bb1 protein does not accumulate in the environment (e.g., air, soil, or water) or in animal tissues. Therefore, non-target soil organisms will be minimally exposed to the Cry3Bb1 protein based on its rapid degradation in the soil.

In summary, Cry3Bb1 poses less risk to the environment than tefluthrin, terbufos, chlorpyrifos, fipronil, or any other conventional insecticide labeled for CRW control. MON 863 corn poses minimal risk to non-target organisms. The Cry3Bb1 protein is expressed by the corn plant, thus reducing the exposure to non-target organisms. In addition, Cry3Bb1 has a narrow target range. Monsanto has performed dietary bioassays to determine the insecticidal spectrum of the Cry3Bb1 protein. The protein is effective at killing only beetles of the family Chrysomelidae, specifically CRW and Colorado potato beetle (*Leptinotarsa decemlineata* (Say)). There have been no functional receptors for Cry proteins found on intestinal cells of fish, birds, or mammals. Finally, Cry3Bb1 is degraded rapidly in the soil (reducing non-target exposure). Use of this new pesticide could potentially reduce the use of CRW chemical pesticides by millions of pounds per year and substantially reduce non-target organism risk.

d. Practical, Easier, and Safer for Growers to Use Than Current Alternatives (MRID Nos. 456530-01 and 450297-01)

MON 863 offers many more practical advantages to corn growers than the current alternatives. It can be planted early for a longer growing season and potentially higher yield, while ensuring adequate CRW protection throughout the growing season. Planting corn early is desirable to boost yield, but it can also reduce insecticide performance because of chemical dissipation prior to larval hatch. With MON 863 corn, the grower can plant early and not have to worry about timing or chemical dissipation. In addition, growers should be able to plant their crop quicker because they won't have to continually stop and refill the insecticide boxes. MON 863 seeds can also have seed treatments that will allow even greater control of other associated pests such as wireworm, grub, maggots, and cutworms. Thus, growers will have multi-pest protection while carrying out insect control in essentially a single step at planting. All of these advantages to planting MON 863 corn are practical, easier, and safer for the grower. Planting MON 863 corn will save the grower money in application, insecticide, labor, fuel, equipment, storage, and disposal costs (since there will be no insecticide containers needed for CRW control). Plus, it will provide the grower and other occupational workers greater safety, protect water bodies from run-off, and mitigate spray drift and non-target effects. Grower interest in MON 863 is high, approximately 70% of growers surveyed were either "very interested" or "somewhat interested" in the new CRW trait hybrids (see page 48 of MRID No. 455770-01). In the first few years, however, there will be a limited amount of seeds available, the trait will not be in all corn varieties, and many growers will try out the new technology rather than planting the maximum 80% of corn with MON 863.

Prior to the development of MON 863, the three CRW control methods have been (1) crop rotation, (2) soil-applied insecticides, and (3) limited use of rescue-treatments for CRW adult beetles. Historically, crop rotation has been the primary method used for controlling CRW (Levine and Oloumi-Sadeghi 1991). Crop rotation, however, is now far less effective because of the existence of a WCRW soybean

rotational variant, primarily in Eastern Illinois and Western Indiana, that oviposits in soybean fields (Levine and Oloumi-Sadeghi 1991) and a NCRW extended diapause (2 year extended) variant, primarily in parts of Minnesota, Iowa, and South Dakota (Krysan *et al*. 1986). In addition, CRW has developed resistance to methyl parathion and carbaryl, both adulticides used in rescue treatments (Meinke *et al*. 1998). Therefore, growers have become increasingly dependent on chemical pesticides to limit CRW losses. MON 863 CRW-protected corn offers a way to potentially control CRW behavioral variants and insecticide-resistant populations more effectively than through the use of chemical pesticides for CRW and still utilize effective corn-soybean (or other crop) rotations. MON 863 corn will likely reduce or eliminate the use of certain CRW insecticides (see discussion below).

MON 863 gives growers equal or higher yields than use of chemical pesticides, while requiring less input of time and other resources. Preliminary results put this yield benefit at 1.5–4.5% (see MRID No. 456430-02). For a reasonable range of prices and yields, the value of this yield benefit to growers is $4–$12/acre relative to the use of soil-applied insecticides and depending on the CRW pressure.

Farmers were surveyed (see MRID No. 456923-01) to determine major factors that would be important to them in deciding whether to plant transgenic corn with CRW resistance, such as MON 863, in place of their current corn. In addition to economic considerations, the farmers indicated the following non-monetary benefits would also be important:

- Safety of not handling a toxic insecticide
- Easy to use and handle
- All-in-one-product insect control
- Saving time and labor
- Better pest control

Farmers were especially interested in minimizing health and environmental effects of the pesticides they use and, if cost and performance are comparable, prefer a general-use product over a restricted-use product. Again, the survey (see MRID No. 456923-01) indicates that farmers will favor the pesticide that minimizes adverse effects on the environment.

e. Efficacy of Event MON 863 (MRID Nos. 453613-03 and 455382-08)

Based on the review of the submitted field efficacy studies, MON 863 corn is as effective or more effective than chemical insecticides in protecting corn roots from larval CRW feeding damage. Chemical pesticides for CRW are usually applied to the soil at the time of planting; however, the pesticide may dissipate and no longer be effective by the time the larvae hatch. Timing is not a problem with MON 863 corn because the pesticide is incorporated within the corn roots and is produced at a relatively constant rate in growing corn. Weather is unlikely to effect the efficacy of MON 863 corn as much as it might decrease the effectiveness of the chemical insecticides. Based on the results discussed below, the extent of root damage sustained by MON 863 was less than that seen in the control corn, and

less than or equal to the damage in corn treated with any of the other chemical pesticides used in the comparative analysis.

i. Comparing the Efficacy of MON 853 and MON 863 to Three Corn Rootworm Species, Northern Corn Rootworm (*D. barberi*), Southern Corn Rootworm (*D. undecimpunctata howardi*), and Western Corn Rootworm (*D. virgifera virgifera*) (MRID No. 455382-08)

In this experiment, Monsanto compared the relative efficacy of two transformed corn hybrids, expressing the Cry3Bb1 protein (MON 853 and MON 863), in preventing damage from three species of corn rootworm larvae. This was accomplished by artificially infesting potted corn plants (treatments consisting of the two transformed hybrids and a non-transformed control hybrid) with eggs from each of three rootworm species. Each plant (in the V2 stage) was infested with approximately 800 eggs (6–8 plants per treatment were used). Root damage was scored using the Iowa Root Damage Rating (RDR) index (1 = no damage, 6 = extensive damage) after 3–4 weeks of larval feeding.

Results from the study showed that both MON 853 and MON 863 experienced significantly less root damage from all three rootworm species than the non-transformed control hybrid. In terms of western and northern corn rootworm damage, MON 863 had significantly less root damage (<2 RDR) than MON 853 (~2.3 RDR). For southern corn rootworm, there was no significant difference between MON 853 and MON 863 (RDR ~3.5–3.8). Southern corn rootworm damage was greater than western or northern corn rootworm damage for all treatments. It is noted that Monsanto is only commercializing Event MON 863.

ii. Efficacy of MON 863 Against Corn Rootworm and Comparison to Insecticide Treatments – Results of Year 2000 Field Trials (MRID No. 453613-03)

In this experiment, Monsanto evaluated the relative effectiveness of MON 863 and conventional pesticide treatments at preventing damage from corn rootworm feeding in field efficacy trials. The pesticides tested (all soil insecticides) included Force 3G (tefluthrin), Counter CR (terbufos), and Lorsban 15G (chlorpyrifos).

The study consisted of three separate field experiments, all of which utilized similar growth stage MON 863 and non-transgenic control hybrids (negative MON 863 isoline). In each of the experiments, treatments were deployed using a randomized block design and were scored for root damage in late July. Root damage was assessed using the Iowa Root Damage Rating index (1 = no damage, 6 = extensive damage, >3 = economic threshold). For the first experiment (conducted at seven different locations), treatments (MON 863, control, Force 3G, Counter CR, and Lorsban 15G) were deployed as four-row strips (4 replicates per treatment). Each plot was artificially infested with 800 rootworm eggs/foot (species not specified). In the second experiment (conducted at eight different locations), MON 863 was evaluated against Force 3G treatment and an untreated control. Treatments were deployed as single rows and were artificially infested with 1,600 rootworm eggs/foot (species not specified). In the third experiment (conducted at nine test sites), treatments (MON 863, control, Force 3G, Counter CR, and

Lorsban 15G) were planted in four-row strips in continuous corn acres or a corn/pumpkin trap crop (no artificial rootworm infestation was used). For all tests, RDR damage was analyzed via analysis of variance and t-tests to determine significant differences between treatments. Also, a "consistency rating" was calculated for each experiment by determining the percentage of root damage in a treatment that is below the economic threshold (RDR = 3) when the corresponding control treatment root damage is above the threshold.

The results of the first experiment showed that when summed across all test locations, MON 863 (RDR = 2.02), Force 3G (RDR = 2.40), Counter CR (RDR = 2.26), and Lorsban 15G (RDR = 2.40) experienced significantly less root damage than the untreated control (RDR = 3.91), although there was no significant difference between MON 863 and the insecticide treatments. At three of the seven locations, MON 863 had significantly less root damage than all of the other insecticide treatments. For the second experiment, when summed across all eight test sites, MON 863 (RDR = 1.41) and Force 3G treatment (RDR = 1.91) showed significantly less root damage than the untreated control (RDR = 3.27). There was no significant difference between MON 863 and Force 3G, although root damage for MON 863 was significantly less than that for Force 3G at five of the test sites. In the third experiment, MON 863 experienced significantly less root damage (RDR = 1.72, summed over all nine locations) than any of the insecticide treatments or the control (all insecticide treatments had significantly less damage than the control). For all three experiments, the "consistency rating" for MON 863 was close to 100%, meaning that damage in the MON 863 hybrids was almost always kept below the economic threshold when the control treatment showed damage exceeding the threshold.

Taken together, the results show that MON 863 prevented root damage from rootworm feeding as well or better than rootworm soil insecticides. Root damage ratings for MON 863 were typically between 1.2 and 2.0, a high level of control relative to untreated control hybrids. In addition, the results were generally consistent from location-to-location (test sites included plots in six separate corn-growing states).

f. Yield Benefits (MRID No. 456530-02)

The field efficacy data discussed above were used to estimate the yield benefit of the MON 863 corn hybrids relative to non-transgenic corn hybrids without corn rootworm control and with a soil insecticide for corn rootworm control (see MRID No. 456530-02). Field data were collected to estimate the proportional yield loss as a function of the root rating difference (1–6 root rating scale of Hills and Peters). Three years of data (1994–1996) in 2 locations in Illinois (near Urbana and DeKalb) were used for the analysis. Data from efficacy experiments conducted in 1999 and 2000 in several locations were used to estimate the impact of Event MON 863 on the root rating. Preliminary estimates, using a composed error model for insect damage functions, indicate that the MON 863 corn hybrids have a yield benefit of 1.5 to 4.5% relative to control with a soil insecticide and 9 to 28% relative to no control. The value of these benefits is estimated to be $4–$12/acre relative to control with a soil insecticide, depending on the corn rootworm pressure, and $25–$75/acre relative to no control. Because there is a low correlation between root rating difference and yield loss, there is uncertainty in the realized yield

benefit. This uncertainty is not due to MON 863 *per se* but to the numerous environmental and agronomic factors determining a corn plant's yield and yield response to corn rootworm larval damage.

g. Grower Benefits (MRID Nos. 456923-01, 456530-01-03, 450297-01, and 455770-01)

Monsanto submitted a study entitled "An Ex Ante Analysis of the Benefits from the Adoption of Monsanto's Corn Rootworm Resistant Varietal Technology - YieldGard® Rootworm." This study examined the potential economic impacts in the U.S. of the commercial adoption of MON 863 corn (YieldGard® Rootworm technology). The model estimates the economic impacts if MON 863 corn had been available and was priced such that the technology fee per acre would be the same as for a representative conventional (non-*Bt*) CRW control technology. The study used data from the year 2000 and made certain assumptions where necessary. For the year 2000, almost 8 million pounds of CRW insecticide, costing $172 million, were applied to 14 million acres (i.e., 17% of total corn acres planted). For a reasonable range of prices and yields, benefit to growers was estimated at $4 to $12/acre, depending on corn rootworm pressure. The authors of the study estimated one-year total benefits (in the year 2000), with 100 percent adoption of MON 863 corn in year 2000, to be $460 million. This benefit includes $171 million to Monsanto and other seed companies, $231 million to farmers from yield gains, $58 million to farmers from reduced risk and time savings, and other benefits associated with the reduced use of insecticides.

i. EPA Projections of Grower Benefits and Environmental Benefits

Grower benefits are a theoretical construct that cannot be directly measured or monitored. They are defined as the premium a grower would pay for MON 863, or the difference between the value of MON 863 and its costs. Grower benefits can be depicted in a graph as the area above the technology fee and below the demand curve. This is where product value, as measured by willingness to pay, exceeds the technology fee. Grower benefit projections are best confirmed by comparing projected adoption rates with actual adoption rates given technology fees.

The factors that will influence grower demand are the following: CRW-infested acres, comparative yields and costs of competing technologies for CRW insect control, U.S. and global market acceptance and approval, and other regulatory constraints (e.g., refuge requirements). About 30% of the corn acreage (24 million acres) was treated with 12 million pounds of insecticides to control pests over the last several years. For the year 2000, almost 8 million pounds of CRW insecticide, costing $172 million, were applied to 14 million acres ($12.29 per acre). The EPA estimates and projections use the submitted comparative performance studies and yield enhancements, which indicate an increased yield of 1.5 to 4.5% for use of MON 863 over chemical pesticides when infestation levels are high.

I. Methodology and Parameter Estimates

The Agency predicted mature market adoption rates based on a demand simulation model and pricing behaviors based on revenue maximization (marginal cost = 0). The demand curve measures adoption at

alternative technology fees for MON 863 corn. A discussion of the simulation model is found in section II(E) EPA's 2001 *Bt* Crops Reassessment (U.S. EPA 2001). Briefly, the distribution of MON 863 perceived grower value and costs are assumed to be uniformly distributed across all infested acreage. Single parameter estimates are required for the maximum product value and cost. The maximum value (willingness to pay) is derived from estimates of (1) improvements to yield, (2) reductions in chemical costs, and (3) the perceived value of a less toxic product. The model also requires an estimate of the negative costs associated with marketability discounts/risks, refuge requirements, or any other costs associated with this technology.

The maximum value reflects the acreage with the highest values due to greatest pest pressures and cost of rootworm control. An estimate of $15.75 is used for the model and is based on a 4.5% yield improvement on $350/acre gross income for corn, including government payments, which is characteristic of expectations for 2001. The cost savings from insecticides includes the out-of-pocket costs of $12.50 per acre plus a maximum of $2.50 per acre due to perceived value as a general use product with less toxic effects to the local environment. The perceived value of a less toxic product is not an out-of-pocket cost and is probably of lesser importance to growers. The high market share for chlorpyrifos (25 % of the CRW market) may be due, in part, to the fact that it is only major alternative registered for general use (i.e., no restricted uses).

Value due to product performance (yield)	$15.75
Value from lower chemical costs	$12.50
Value from easier and safer use	$ 2.50
Max value of *Bt* corn rootworm	$30.75

It is unlikely, based on past experience with biotechnology products, that European Union approval will occur in less than three years after launch (see MRID No. 455770-01). This international regulatory constraint imposes an additional cost on adoption of MON 863 corn. Just as was done for Roundup Ready® corn, until full European (or global) regulatory approval occurs, Monsanto plans to continue its channeling program with growers, dealers, and grain handlers to help ensure that MON 863 corn is directed into appropriate global markets (markets with regulatory approval). The simulation model can be used to assess the impact of access to global markets. The negative costs associated with limited marketability are reflected by the percent of growers who would not use MON 863 even if there was no technology fee. That is, adoption would not be 100% even if MON 863 is given away. Based on the results of a survey on grower attitudes toward genetically modified organisms (conducted by the American Corn Growers Association), sixteen percent of respondents stated they would not be willing to grow more non-genetically engineered corn varieties (see MRID No. 456923-01). The 16% of growers is consistent with a maximum cost of $10 per acre as compared to a maximum cost of $5 per acre, the 9% (91% adoption) at $0/acre technology fee. A 16% removal from the target market would reduce adoption rates from 43% to 35%, which would translate to lower grower benefits and less use reduction.

The demand curve and derived marginal revenue curve provide a basis for predicting a technology fee. The estimate of $15 per acre technology fee is based on revenue maximization behaviors that are

equivalent to profit maximization if marginal cost is zero. The actual technology fee would vary from this estimate based on licensed seed companies' perceptions of the demand curves for specific hybrids and marginal costs associated with marketing and sales.

EPA's economic assessment and eleven (11)-year projection of aggregate grower benefits is based on current chemical prices of alternatives. It neither anticipates nor includes any price changes from competing technologies as MON 863 corn is introduced or the effects of new active ingredients registered for corn rootworm. The economic assessment is limited to grower benefits and does not estimate the reduced cost passed through to final consumers (though in the long run, economic theory suggests that the 2% improvement in returns on gross revenue would be passed through to consumers). No assessments are made of impacts to foreign trade or agricultural practices. MON 863 corn may lower the costs of rootworm control and, therefore, have some effect on acres grown to continuous corn, which would increase pest pressures and reduce the environmental benefits of MON 863 (UCS 2002).

II. Corn Rootworm-Infested Acres

Scouting for the range and level of infestation is done by measuring the density of adult beetles and larvae. Gray (2000) noted that CRW-infested acres are increasing due to the geographical expansion of the WCRW soybean variant. Based on both the likely geographic spread of the WCRW soybean variant and the NCRW extended diapause variant, it is likely the total infested acres will move from approximately 28 million acres to closer to 39 million acres in 13 years. This assessment projects the range of infestation to increase uniformly by 2.6% per year, and the density to remain such that only ½ of the acreage infested is above the economic threshold. To the extent that market forces reduce the economic threshold (increased corn prices, increased yields, or reduced cost of CRW control), acreage adoption and conventional insecticide use would be higher than currently forecast, and the environmental and grower benefits of MON 863 would also be higher than projected in this review.

EPA has considered the rate of increase of CRW-infested aces as input into its simulation model that was used to predict the technology fee, adoption rates, and grower benefits of MON 863 corn.

III. MON 863 Corn Hybrid Supply

Adoption of MON 863 corn (acres) is dependent on supply and demand. The supply is constrained by corn seed hybrid availability, both for a single hybrid and the total number of hybrids available. The commercial hybrid development process requires sequential development that will take several years after commercial launch.

In the first two to three years after commercial launch, adoption of MON 863 corn is predicted to be relatively slow because there will only be a limited number of MON 863 corn seed hybrids available. Monsanto has projected that MON 863 corn adoption will be similar to Roundup Ready® corn in that it will not be available in Pioneer or Syngenta brands nor have European Union approval at the time of launch (see MRID No. 455770-01). Roundup Ready® corn had approximately 1% (790,000 acres),

2.5% (2 million acres), and 5% (4 million acres) acreage penetration in percent of total corn acres in years 1, 2, and 3 from commercial release, respectively. These data were used by EPA in its economic analysis.

IV. Estimating the Demand Curve for MON 863

The demand curve for MON 863 for year 2013 is shown in Table 7. It is based on a simulation of adoption at alternative technology fees for MON 863 as described in section II(E)(2)(g)(i) of this BRAD. Marginal revenue is computed using a technology fee of $15 based on revenue maximization. It is based on pricing behavior where the marginal costs of increasing seed production are negligible (assumes that all seed hybrids are in place). It is the last point where marginal revenue is positive (see bolded row in Table 7 below). The total revenue with a $15 technology fee is $252 million for 16.9 million acres of MON 863 corn planted in the year 2013. Actual technology fees are certain to vary from this estimate. At a $15 technology fee, MON 863 adoption is predicted to be 43% of infested acreage. If only 50% of infested acreage is treated, then only 7% of infested acreage will still be treated by conventional chemical controls (not including any refuge acres required as part of an insect resistance management plan).

Table 7. Simulated Demand Curve for the Year 2013.

Tech Fee Schedule ($)	Percent Adoption (Model Calculation)	Acres Adopted (X 10^6)	Total Revenue (X 10^6)	Marginal Revenue (X 10^6)	Marginal Revenue Per Acre ($)
27	5%	1.95	52.65		
24	14%	5.46	131.04	78.39	22.33
21	23%	8.97	188.37	57.33	16.33
18	34%	13.26	238.68	50.31	11.73
15	**43%**	**16.77**	**251.55**	**12.87**	**3.67**
12	53%	20.67	248.04	(3.51)	(0.90)
9	63%	24.57	221.13	(26.91)	(6.90)
6	72%	28.08	168.48	(52.65)	(15.00)
3	83%	32.37	97.11	(71.37)	(16.64)
0	91%	35.49	0.00	(97.11)	(31.12)

V. Projecting Grower Benefits

Grower benefits are calculated as the sum of the difference between what the grower is willing to pay and the actual technology fee. The EPA simulation model computes the average gross benefits for adopters and *Bt*-related costs due to MON 863 corn adoption. The estimated net benefits per acre are $6.56, based on a $15 technology fee (see Table 7 above). The estimated gross benefits (primarily, yield and insecticide cost reduction) for adopters are $23.94 per acre, and estimated *Bt*-related costs for adopters are $2.38 per acre.

The annual change in infested acres, adoption, conventional chemical use, and associated grower benefits are projected for each year from 2003 to 2013 (Table 8). Projected acres infested and conventional chemical treatments are based on growth rates for infested acreage with a fixed treatment percentage of 50% of the total projected CRW-infested acres.

Table 8. Projected Acreage Infested, MON 863 Adoption, and Conventional Treatments (2003 to 2013).

Year	Acres Infested ($X 10^6$)	Acres Treated ($X 10^6$)	MON 863 Acres ($X 10^6$)	Conventional Treatments ($X 10^6$)
2000	28.0	14.0	0.0	14.0
2002	29.5	14.7	0.0	14.7
2003	30.2	15.1	1.0	14.1
2004	31.0	15.5	2.5	13.0
2005	31.8	15.9	4.0	11.9
2006	32.6	16.3	6.0	10.3
2007	33.5	16.7	7.2	9.5
2008	34.3	17.2	8.6	8.5
2009	35.2	17.6	10.4	7.2
2010	36.1	18.1	11.9	6.1
2011	37.1	18.5	13.7	4.8
2012	38.0	19.0	15.8	3.2
2013	39.0	19.5	16.8	2.7
Annual Growth Rate	**2.58%**	**2.58%**		**-14.36%**

Annual grower benefits (see Table 9) are based on a constant $6.56 per acre, and the growth in total annual benefits are due to the availability of hybrid seed containing Cry3Bb1 over greater areas of CRW infestation. Actual grower benefits may be higher in the early years, if supply is first available in those areas with highest CRW pest pressure.

The cumulative sum of the grower benefits for the first three years (2003 to 2005) is $49.2 million and for eleven years (2003 to 2013) is $642.7 million (Table 9). The discounted aggregate benefits for year 2003 to 2013 are $385.31 million, assuming a discount rate of 7%. The 7% discount rate represents the Office of Management and Budget rate (see Circular Number A-94, Transmittal Memo Number 64, October 29, 1992), a relatively risk-free rate of return similar to that assumed for a long-term U.S. Treasury bond. The discount rate is defined as the interest rate used in calculating the present value expected yearly for benefits and costs.

Table 9. Annual Grower Benefits of MON 863.

Year	Target Acres ($X 10^6$)	Adoption Acres ($X 10^6$)	Grower Benefits ($\$ X 10^6$)
2003	2.3	1.0	6.6
2004	5.8	2.5	16.4
2005	9.3	4.0	26.2
2006	14.0	6.0	39.4
2007	16.7	7.2	47.2
2008	20.1	8.6	56.7
2009	24.1	10.4	68.0
2010	27.7	11.9	78.2
2011	31.9	13.7	90.0
2012	36.7	15.8	103.5
2013	39.0	16.8	110.5
Cumulative		**98.0**	**$ 642.7**

VI. Projecting Chemical Use with MON 863 (MRID Nos. 456530-01 and 450297-01)

The insecticides used to control corn rootworm in conventionally grown (non-*Bt*) corn consist mainly of organophosphates (9), carbamates (3), synthetic pyrethroids (6), and phenyl pyrazole (1) classes of chemistry (see Table 1). Table 10 shows that there have been significant shifts in the use of insecticides. Synthetic pyrethroids have increased at the expense of organophosphates and carbamates. The clear shift is away from organophosphate insecticides and toward synthetic pyrethroids, especially the effective, relatively new product tefluthrin that is now the market leader. Fipronil was introduced in 1998 and accounts for the other category. Table 11 projects future acre treatments, by chemical class, using past trends and the projected conventional treatments shown in Table 8.

Table 10. Historical Market Shares for Corn Rootworm (Percent of Total Acre Treatments).

Chemical Class	1995	2001
Carbamate	4.5%	2.1%
Synthetic Pyrethroid	21.9%	41.0%
Organophosphate	73.4%	45.8%
Other	0.1%	11.0%
Total	100.0%	100.0%

Table 11. Projected Treatments for Corn Rootworm (Millions of Acre Treatments for Corn Rootworm).

Chemical Class	2002	2003	2004	2005	2006	2007
Carbamate	0.3	0.3	0.2	0.2	0.2	0.1
Synthetic Pyrethroid	6.2	6.2	5.9	5.6	5.0	4.8
Organophosphate	6.5	6.0	5.3	4.6	3.8	3.3
Other	1.7	1.7	1.6	1.5	1.4	1.3
MON 863	0.0	1.0	2.5	4.0	6.0	7.0
Total CRW-Treated	14.7	15.1	15.5	15.9	16.3	16.5

Table 12. Projected Use Reduction Associated with MON 863 (Millions of Acre Treatments for Corn Rootworm)

Chemical class	2002	2003	2004	2005	2006	2007
Carbamate	0.0	0.0	0.0	0.1	0.1	0.1
Pyrethroid	0.0	0.4	1.1	1.9	2.9	3.5
Organophosphate	0.0	0.4	1.0	1.5	2.2	2.5
Other	0.0	0.1	0.3	0.5	0.8	0.9
total reduction	0.0	1.0	2.5	4.0	6.0	7.0

These pesticides have varying application rates, many are toxic to humans and non-target wildlife, and many have restricted use or specific, mandatory mitigation measures to minimize exposure. The average active ingredient rate applied (pounds per acre) has been steadily decreasing, reflecting the shift from organophosphates and carbamates to synthetic pyrethroids and fipronil. Rates have gone from an average of 0.7 pounds per acre in 1995 to 0.4 pounds per acre in 2001. The application rates shown in Table 1 for terbufos, chlorpyrifos, carbofuran, tebupirimfos, and phorate are closer to 1 pound per acre. Synthetic pyrethroids and fipronil, for example, are newer chemistries that are used at 0.1 (or less) pound per acre. The use reductions shown in Table 12 indicate that, as MON 863 CRW-protected corn adoption increases in the next five years, acre treatments will be reduced for all currently registered CRW insecticides. The greatest use reductions are seen in both the organophosphate and synthetic pyrethroid classes. In 2005, approximately 1.5 million acre treatments of organophosphate insecticides, 1.9 million acre treatments of synthetic pyrethroid insecticides, 0.1 million acre treatments of carbamate insecticides, and 0.5 million acre treatments of other chemical insecticides, including members of the phenyl pyrazole class (e.g., fipronil), will be reduced based on 2003 figures. In 2007, the extent of insecticide use reduction will be even greater, approximately 2.5 million acre treatments of organophosphate insecticides, 3.5 million acre treatments of synthetic pyrethroid insecticides, 0.1

million acre treatments of carbamate insecticides, and 0.9 million acre treatments of other chemical insecticides are expected to be reduced.

ii. Comparing Estimates of Grower Benefits from Other Studies

Reported estimates may differ with respect to the entities included and target year. Some include the grower and chemical producer and reflect the total societal value of MON 863. EPA's assessment is limited to grower benefits. Estimates made in MRID No. 456923-01 are based on an ex ante assessment, assuming that MON 863 was available in the year 2000. It is necessary to adjust estimates to the extent possible to create valid comparisons.

The authors of the study (MRID No. 456923-01) estimated one-year total benefits (in the year 2000), with 100 percent adoption of MON 863 corn in year 2000, to be $460 million. This benefit includes $171 million to Monsanto and other seed companies, $231 million to farmers from yield gains, $58 million to farmers from reduced risk and time savings, and other benefits associated with the reduced use of insecticides. The EPA assessment for year 2013 is based on a higher level of infested acreage. Adjusting the analysis (from MRID No. 456923-01) of the ex ante total benefits for the year 2000 of $460 million to the year 2013 by an additional 39% gives $640 million in total benefits.

The EPA estimate of total benefits can be calculated assuming a zero technology fee. This is essentially the total area under the demand curve. Total benefits would be $507 million in 2013. This compares with the adjusted estimate of $640 million total benefits mentioned in the previous paragraph.

In a separate analysis, Gray (2000) states that if farmers invested $400 million in this technology (technology fees are assumed to $15 per acre), these resources would prevent an economic loss of approximately $600 million, for a net gain of $200 million to farmers. Adjusting this estimate to coincide with infestation levels in 2013 (a 39% increase) provides total benefits of $834 million.

EPA's estimate of total benefits in 2013 ($507 million) is 20% lower than projections from MRID No. 456923-01 and 40% lower than Gray (2000)'s projections.

h. Suggested Measures to Monitor Environmental and Grower Benefits

The amount of chemical use reduction attributed to MON 863 cannot be directly observed. For example, if the economic threshold for CRW treatment is reduced by the increased competition created by MON 863, then total infested acres treated would increase. The effect of MON 863 on chemical use reduction would be less then 1 acre per adopted acre of MON 863. A survey of growers who adopted MON 863 would be helpful to directly estimate chemical use reduction.

Translating the environmental benefits of chemical use reduction is a topic that is given increased attention for the purpose of strategic planning and measuring results. Measures most likely affected by the reduction in insecticide use are reported incidents from workers, accidental spills, and mortality to non-target wildlife.

EPA's Environmental Fate and Effects Division in the Office of Pesticide Programs has identified the most toxic active ingredients to birds, based on risk assessments of all available information. The list contains 10 insecticides currently being used on agricultural crops: aldicarb, methyl parathion, dicrotophos, carbofuran, phorate, oxamyl, diazinon, disulfoton, methamidophos, and ethoprophos. Methyl parathion, carbofuran, phorate, diazinon, and ethoprophos are active ingredients registered as formulated products for the control of corn rootworm (i.e., alternatives to MON 863). The use of methyl parathion, carbofuran, and phorate for corn rootworm control accounts for 1.2 million acres—6.6% of the total use of the ten insecticides on all agricultural crops. Thus, MON 863 alone can have a significant impact on reducing the use of insecticides posing the highest risk to birds. Data are not currently available to estimate impact exactly, but additional data may be collected during the time of the MON 863 conditional registration that would be useful in determining the impact of *Bt* corn on birds and bird populations (e.g., percent of invasive species, native species of management concern, changes in types of and/or abundance of bird species, etc.).

3. 2005 MON 88017 and MON 88017 x MON 810 Public Interest Finding (Reviewed in U.S. EPA 2005)

MON 88017 (plasmid vector ZMIR39) expresses the Cry3Bb1 *Bt* toxin and is targeted against corn rootworm larvae. The toxin is the same as expressed by MON 863 corn (EPA Reg. No. 525-528), registered by Monsanto for the 2003 growing season. The Cry3Bb1 protein produced in MON 88017 and MON 863 is a variant of the wild-type Cry3Bb1 protein from *Bt* subsp. *kumamotoensis*. When compared by amino acid sequencing, the Cry3Bb1 protein expressed in MON 88017 has been reported to be 99.8% similar to the Cry3Bb1 protein expressed in MON 863. The primary difference between the two hybrids is that MON 88017 also expresses a gene for resistance to glyphosate-based herbicides.

Given the similarities between MON 863 and MON 88017, Monsanto proposed to bridge the public interest finding that was made for MON 863 (see section II(E)(2) of this BRAD) to the MON 88017 and MON 88017 x MON 810 registrations. The MON 863 benefits assessment concluded that the registration of MON 863 would be in the public interest.

Monsanto identified the following benefits of the MON 863 registration that should also be applicable to the MON 88017 and MON 88017 x MON 810 registrations:

- Replacement of higher risk pesticides
- Practical benefits for growers (reduced input costs, time and labor savings, etc.)
- Efficacy (equivalent to conventional insecticides)
- Human health benefits (reduced toxicity relative to conventional insecticides)
- Environmental benefits (reduced risks to non-target organisms relative to conventional insecticides)
- Yield benefits (greater yields than conventional corn)

- Grower economic benefits (total benefits up to $6.56 per acre)

Monsanto also identified several benefits that MON 88017 offers in addition to the MON 863 benefits listed above:

- Herbicide tolerance – MON 88017 (and MON 88017 x MON 810) have been engineered to tolerate glyphosate herbicide applications. This trait will provide economic benefits to growers.

- Enhanced breeding efficiency – MON 88017 can be bred faster (and selected through the herbicide tolerance trait), which should allow for a greater supply of the product to growers.

The Biopesticides and Pollution Prevention Division (BPPD) agrees with Monsanto's rationale to bridge the public interest finding for MON 863 to the MON 88017 and MON 88017 x MON 810 registrations. The benefits identified for MON 863 (as summarized above and discussed more comprehensively in section II(E)(2) of this BRAD) are all applicable to the MON 88017 products. In addition, MON 88017 and MON 88017 x MON 810 offer further benefits with the addition of herbicide (glyphosate) tolerance. These factors address the criteria established in the Federal Register Notice dated March 5, 1986 (51 FR 7628) in terms of need, comparative risk issues, and comparative benefits. As such, the registration of both MON 88017 and MON 88017 x MON 810 can be expected to be in the public interest.

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III. REGULATORY POSITIONS FOR CORN EVENT MON 863 AND MON 88017

A. Initial Registration (February 24, 2003) – Corn Event MON 863

Pursuant to section 3(c)(7)(C) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Environmental Protection Agency (EPA) may conditionally register a new pesticide active ingredient for a period of time reasonably sufficient for the generation and submission of required data that are lacking because insufficient time has elapsed since the imposition of the data requirement for those data to be developed. EPA may grant such conditional registration only if EPA determines that (1) the use of the pesticide product during the period of the conditional registration will not cause any unreasonable adverse effect on the environment, and (2) the registration and use of the pesticide during the conditional registration is in the public interest. EPA determines that all of these criteria have been fulfilled.

The first criterion under FIFRA section 3(c)(7)(C), as mentioned above, has been met because insufficient time has elapsed since the imposition of the requirements for the following data:

1. Independent laboratory analytical method validation.
2. Cry3Bb1 protein expression data in terms of dry weight, as the amount of protein present in the given tissue.
3. Field degradation studies evaluating accumulation and persistence of Cry3Bb1 protein in several different soils in various strata.
4. Laboratory toxicity test with *Orius insidiosus* (minute pirate bug).
5. Laboratory toxicity test with carabid (ground beetle).
6. Laboratory toxicity test with *Tetraopes* (red milkweed beetle).
7. Intermediate and multi-year non-target organism field studies with statistical power.
8. A six-week broiler dietary study.
9. Research regarding corn rootworm adult and larval movement and dispersal, mating habits, ovipositional patterns, number of times a female can mate, and fecundity.
10. Research to determine if insect resistance management (IRM) strategies designed for western corn rootworm (WCRW) and northern corn rootworm (NCRW) are appropriate for Mexican corn rootworm (MCRW).
11. Research regarding the mechanism of potential resistance of corn rootworm (CRW) to MON 863. Monsanto must attempt to develop resistant CRW colonies to aid in determining selection intensity.
12. Research regarding the effect of WCRW ovipositing in soybean prior to overwintering and extended diapause in NCRW on an IRM strategy.
13. Detailed summaries of the four data-sets identified in Monsanto's December 13, 2002 letter should be submitted to the Agency to support their conclusion that the initial resistance allele frequency is ≤0.01.
14. Continuation of baseline susceptibility studies, currently underway for WCRW, and initiation for NCRW and monitoring techniques, such as discriminating dose concentration assays, as well as investigation of their feasibility as resistance monitoring tools.

The registration applicant has submitted or cited data sufficient for EPA to determine that a conditional registration under FIFRA 3(c)(7)(C), for the period ending May 1, 2004, will not result in unreasonable adverse effects on the environment. The human health effects data and non-target organism effects data are considered sufficient for the period of the conditional registration. These data demonstrate that no foreseeable human health hazards or ecological effects are likely to arise from the use of the product and that the risk of resistance developing to *Bacillus thuringiensis* Cry3Bb1 protein during the conditional registration is not expected to be significant. The data also demonstrate that there is virtually no possibility of any risk associated with weediness or outcrossing to wild relatives.

Registration of *Bacillus thuringiensis* Cry3Bb1protein and the genetic material necessary for its production (vector PV-ZMIR13L) in event MON 863 corn is in the public interest because MON 863 (YieldGard® Rootworm) corn is less risky to human health and/or the environment than currently registered pesticides and the improved season-long protection and practical benefits of ease of MON 863 use exceed those of the currently registered alternatives, most of which are restricted-use products.

Specifically, MON 863 is in the public interest for the following reasons:

(1) For the first 3 years, MON 863 is projected to reduce conventional pesticide use by 12.5 million pounds of active ingredient over 7.5 million corn acres (0.1 carbamates, 3.5 pyrethroids, 3.0 organophosphates, and 0.9 for other chemical classes). This totals to 7.5 million acres of use reduction. To the extent that MON 863 is used on acreage that would be uneconomical to otherwise treat, the total use reduction would be less than the MON 863 acres adoption.

(2) It has a predicted yield benefit of 1.5–4.5% per acre greater than conventionally treated corn.

(3) Grower benefits are estimated at $6.56 per acre vs. conventionally treated corn.

(4) The total first 3 year economic benefits are estimated at $49.2 million.

(EPA's public interest analysis considers three years of MON 863 use because EPA has been informed that Monsanto will request that the current tolerance exemption for Cry 3bB1, which expires on May 1, 2004, be amended to remove the expiration date. If (1) Monsanto requests such an amendment to the Cry3Bb1 tolerance exemption, (2) EPA grants such amendment request, and (3) Monsanto subsequently requests that the MON 863 registration be amended to expire at a later date, EPA currently believes that the data reviewed so far will likely support an extension of the conditional registration for an additional two years.)

In view of these minimal risks and the clear benefits related to YieldGard® Rootworm, EPA believes that the use of the product during the limited period of the conditional registration will not cause any unreasonable adverse effects.

Although the data with respect to this particular new active ingredient are satisfactory, they are not sufficient to support an unconditional registration under FIFRA 3(c)(5). Additional data are necessary to evaluate the risk posed by the continued use of this product. Consequently, EPA is imposing the data requirements specified earlier in this chapter.

EPA has determined, as explained in section II(E) of this Biopesticides Registration Action Document (BRAD), that the third criterion for a FIFRA 3(c)(7)(C) conditional registration has been fulfilled because the use of Cry3Bb1 corn under this registration would be in the public interest.

A tolerance exemption has been granted, pursuant to section 408(d) of the Federal Food, Drug, and Cosmetic Act (FFDCA), for *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production in corn. This tolerance exemption expires in May 2004 and, therefore, this registration would also expire in May 2004 until such time as the tolerance exemption is amended to extend its duration. If the tolerance exemption is amended to extend its duration, EPA believes that current data and information reviewed would support an amendment of the conditional registration to expire in three years from the date of the original registration.

Conclusion

The submitted data in support of this registration under section 3(c)(7)(C) of FIFRA have been reviewed and determined to be adequate. Studies mentioned previously are included in the terms, conditions, and limitations of this registration. EPA determines that, for the period of conditional registration, this registration will not cause unreasonable adverse effects to man or the environment and is in the public interest.

Based on the data submitted and cited by the applicant and reviewed by Biopesticides and Pollution Prevention Division, EPA has concluded that Monsanto Company's Cry3Bb1 corn product, containing the new active ingredient *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector PV-ZMIR13L) in Event MON863 corn, be REGISTERED under FIFRA section 3(c)(7)(C), with appropriate limitations.

B. 2010 Update – Corn Event MON 863 (and MON 863 x MON 810)

Monsanto did not request an extension to their Corn Event MON 863 (EPA Reg. No. 524-528) or MON 863 x MON 810 (EPA Reg. No. 524-545) registrations; therefore, these registrations expired on their own terms on September 30, 2010. The Agency considers the expiration of a conditional, time-limited registration to be a cancellation under Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). A cancellation order, effective September 30, 2010, and appropriate provisions for disposition of existing stocks published in the Federal Register on August 25, 2010 (75 FR 52329).

C. Initial Registration (December 15, 2005) – MON 88017 and MON 88017 x MON 810

Pursuant to section 3(c)(7)(C) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Environmental Protection Agency (EPA) may conditionally register a new pesticide active ingredient for a period of time reasonably sufficient for the generation and submission of required data that are lacking because insufficient time has elapsed since the imposition of the data requirement for those data to be developed. EPA may grant such conditional registration only if EPA determines that (1) the use of the pesticide product during the period of the conditional registration will not cause any unreasonable adverse effect on the environment, and (2) the registration and use of the pesticide during the conditional registration is in the public interest. EPA determines that all of these criteria have been fulfilled.

EPA determined that it is appropriate to conditionally register the MON 88017 products under FIFRA section 3(c)(7)(C) for a period of time (specified below) reasonably sufficient for the generation and submission of certain data that are lacking because insufficient time has elapsed since the imposition of those data requirements:

(1) For MON 88017, all data that was previously required as a condition of registration to support the individual plant-incorporated protectant in Event MON863 (YieldGard® Rootworm), 524-528, are necessary. In the event that the Agency concludes that the MON 863 (YieldGard® Rootworm) studies required in connection with the MON 863 conditional registration do not sufficiently demonstrate a lack of significant adverse effects, additional data with MON 88017 corn must be submitted. These data may include the following: (a) laboratory toxicity testing with *Orius insidiosus* (minute pirate bug), (b) laboratory toxicity testing with a carabid (ground beetle), (c) long-range effects testing on invertebrate populations in the field, and (d) long-range soil persistence testing.

(2) For MON 88017 x MON 810, all data required to support the individual plant-incorporated protectants in MON 810 (YieldGard®), Event MON 863 (YieldGard® Rootworm), MON 88017 corn; EPA Registration Nos. 524-489, 524-528, are necessary. In the event that the Agency concludes MON 863 (YieldGard® Rootworm) studies do not sufficiently demonstrate a lack of significant adverse effects, additional data with MON 88017 x MON 810 corn must be submitted. These data may include (a) laboratory toxicity testing with *Orius insidiosus* (minute pirate bug), (b) laboratory toxicity testing with a carabid (ground beetle), (c) long-range effects testing on invertebrate populations in the field, and (d) long-range soil persistence testing. Additionally, expression level data regarding Cry1Ab protein levels in MON 810 and MON 88017 x MON 810 young root and forage root are required within 12 months of the date of registration.

At this time, the applicant has submitted or cited data sufficient for EPA to determine that conditional registration of *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector PV-ZMIR39) in MON 88017 corn (OECD Unique Identifier: MON-88Ø17-3) under FIFRA 3(c)(7)(C) will not result in unreasonable adverse effects to the environment during the period of conditional registration, as discussed previously. Monsanto submitted and/or cited satisfactory data pertaining to the proposed use. The human health effects data and non-target organism effects data are

considered sufficient for the period of the conditional registration. These data demonstrate that no foreseeable human health hazards or ecological effects are likely to arise from the use of the products during the period of conditional registration and that the risk of resistance developing to Cry3Bb1 proteins during the conditional registrations are not expected to be significant.

Registration of MON 88017 and MON 88017 x MON 810 is in the public interest for the following reasons:

- Replacement of higher risk pesticides.
- Practical benefits for growers (reduced input costs, time and labor savings, etc.).
- Efficacy (equivalent to conventional insecticides).
- Human health benefits (reduced toxicity relative to conventional insecticides).
- Environmental benefits (reduced risks to non-target organisms relative to conventional insecticides).
- Yield benefits (greater yields than conventional corn).
- Grower economic benefits (total benefits up to $6.56 per acre).
- Herbicide tolerance: MON 88017 (and MON 88017 x MON 810) have been engineered to tolerate glyphosate herbicide applications. This trait will provide economic benefits to growers.
- Enhanced breeding efficiency: MON 88017 can be bred faster (and selected through the herbicide tolerance trait), which should allow for a greater supply of the product to growers.

In view of these minimal risks and the clear benefits related to MON 88017 (in both the MON 88017 and MON 88017 x MON 810 products), EPA believes that the use of the products, during the limited period of the conditional registration, will not cause any unreasonable adverse effects.

Although the data with respect to this particular new active ingredient are satisfactory, they are not sufficient to support an unconditional registration under FIFRA 3(c)(5). As noted above, additional data are necessary to evaluate the risk posed by the continued use of these products. Consequently, the Agency has imposed the data requirements specified earlier in this chapter.

Permanent tolerance exemptions exist and are applicable to the MON 88017 products, MON 88017 and MON 88017 x MON 810.

Based on the data submitted and cited by the applicant and reviewed by EPA, the EPA registered Monsanto's Cry3Bb1 MON 88017 corn products, containing the new active ingredient *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector PV-ZMIR39) in MON 88017 corn (OECD Unique Identifier: MON-88Ø17-3), be REGISTERED under FIFRA section 3(c)(7)(C), with appropriate limitations.

The expiration date of the registration has been set to September 30, 2010 for MON 88017 and October 15, 2008 for MON 88017 x MON 810 (due to the MON 810 2008 expiration date).

D. 2010 Update – MON 88017 and MON 88017 x MON 810

Section 3(c)(7)(A) of FIFRA provides for the registration or amendment of a pesticide when the pesticide and proposed use "…are identical or substantially similar to any currently registered pesticide and use thereof, or differ only in ways that would not significantly increase the risk of unreasonable adverse effects on the environment, and (ii) approving the registration or amendment in the manner proposed by the applicant would not significantly increase the risk of any unreasonable adverse effect on the environment." Unreasonable adverse effects on the environment are defined under section 2(bb) of FIFRA as "… any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide…" Thus, pursuant to section 3(c)(7)(A), EPA may conditionally register a pesticide if (1) the pesticide and its proposed use are identical or substantially similar to a currently registered pesticide; or (2) the pesticide and its proposed use differ only in ways that would not significantly increase the risk of unreasonable adverse effects; and (3) approving the registration would not significantly increase the risk of any unreasonable adverse effect.

The Agency concludes that the following Cry3Bb1 corn product registrations, set to expire on September 30, 2010 and described in-depth throughout this BRAD, meet both criteria (1) and (2):

(1) MON 88017 (EPA Reg. No. 524-551)
(2) MON 88017 x MON 810 (EPA Reg. No. 524-552)

These Cry3Bb1 corn products are identical in both composition and use (corn) to plant-incorporated protectants that are currently registered. Thus, criterion (1) has been fulfilled.

With regard to criterion (2), the Agency maintains, as was previously determined for the original registration of these particular products, that cultivation of Cry3Bb1-containing corn will not cause unreasonable adverse effects on the environment. The conditional environmental effects data, submitted in response to terms and conditions of registration and summarized in sections II(C)(2)(b), II(C)(3)(b), and II(C)(4)(b) of this BRAD, strengthen the Agency's initial position and also confirm that long-term effects on non-target organisms are not anticipated. Lastly, the continued use of these products will likely still provide many of the benefits as were evaluated in section II(E) of this BRAD to support the 2003 registration of Corn Event MON 863 and the 2005 registrations of MON 88017 and MON 88017 x MON 810 (e.g., reduction in use of conventional insecticides that are highly toxic to both humans and the environment).

In conclusion, as the expiring Cry3Bb1 products have met the required criteria under section 3(c)(7)(A) of FIFRA, the Agency is amending these registrations to extend their respective expiration dates[a] as follows:

Product Name (EPA Reg. No.)	Expiration Date
MON 88017 (524-551)	September 30, 2015
MON 88017 x MON 810 (524-552)	September 30, 2015

Although data provided were satisfactory to make the determinations required by section 3(c)(7)(A) of FIFRA, they were not sufficient to support an unconditional registration under FIFRA section 3(c)(5). Additional data, specifically in relation to insect resistance management, are necessary for a finding of registrability under FIFRA section 3(c)(5) and will remain as terms or conditions for the purposes of the amendments.

[a] See section III(E) of this BRAD for an explanation describing how the proposed expiration dates were determined.

E. Period of Registration

In the 2001 *Bt* Corn reassessment, EPA determined that it was appropriate to amend the then-existing registrations to extend the period of registration of those products to an expiration date of October 15, 2008. All of the products being assessed at that time were efficacious against lepidopteran pests. EPA based this action on the finding that use of Cry1Ab or Cry1F expressed in corn will not significantly increase the risk of unreasonable adverse effects on the environment "for the limited time period of 7 additional years (to October 15, 2008)." These registrations were later amended to extend the period of registration to an expiration date of September 30, 2010. EPA subsequently granted time-limited registrations to products efficacious against coleopteran corn rootworm pests. For example, EPA registered Cry3Bb1 on February 24, 2003, to May 1, 2004, and extended that registration twice, to February 24, 2008, and September 30, 2010.

As set forth elsewhere in this document, EPA's primary concern for the *Bt* protected transgenic corn products is the possibility that target pests will develop resistance to one or more of the plant-incorporated protectant (PIP) toxins. Development of resistance to a *Bt* toxin would be a grave adverse effect, and, for over 15 years, EPA has imposed stringent requirements intended to countermand the potential development of resistance. Registrants similarly have been busily developing various products, product mixes (i.e., so-called "pyramids" and "stacks"), and resistance strategies, to maximize agronomic benefits and address resistance management issues. The result has been a vast array of product combinations and, occurring over the past couple of years, a re-emergence of varying refuge requirements for different products.

As discussed in the 2001 *Bt* PIP BRAD (at IID13), the earliest *Bt* corn registrations did not include mandatory refuge requirements. There was a lack of scientific consensus as to what the appropriate refuge requirement should be, and, it was assumed that the limited market penetration of these early crops would be so low as to guarantee that adequate natural refuges would be available from neighboring non-*Bt* corn fields. From 1995 to 1997, *Bt* corn registrations included voluntary refuge requirements of 0% to 20% in the Corn Belt. In 1999, the Agricultural Biotechnology Stewardship Technical Committee (ABSTC), in conjunction with the National Corn Growers Association, proposed uniform insect resistance management (IRM) requirements for *Bt* corn registrations. With some modifications, this proposal, put in place for the 2000 growing season, formed the baseline IRM requirements for almost all *Bt* corn registrations for the better part of a decade: farmers were required to plant a 20% refuge that could be treated for insects, or a 50% treated refuge in cotton-growing areas; all refuges to be planted within one-half mile of the *Bt* corn field.

These uniform requirements brought certainty and consistency to the market after the initial period where many *Bt* corn products had different refuge requirements. Recently, however, as product developers have begun to conceive of products with different combinations of "pyramided" products (i.e., products containing two or more toxins efficacious against the same pest) and "stacked" products (i.e., products combining toxins efficacious against different pests), the refuge requirements have begun to vary. For example, certain products require a 20% external refuge; some products permit a 5%

external refuge; one product incorporates a 10% seed blend refuge; we have applications in process for products that propose to incorporate a 5% seed blend refuge; and other permutations are possible.

Given the profusion of various toxin combinations and refuge options, we can no longer proceed on the basis that, as concerns insect resistance management, all products are equal. It was a relatively simple proposition when the default requirement of a 20% sprayed refuge applied to almost all of the *Bt* corn crops in the market. Under those circumstances, the relative durability of products against the development of resistance was functionally equivalent, and, as a consequence, imposing functionally equivalent registration periods was appropriate. That is now no longer the case.

As part of our continually evolving regulatory approach to the continually evolving product mix wrought by developers, we think it appropriate to revise our regulatory requirements in scientifically defensible ways to reflect the comparative level of risks posed by the products that we regulate. Here, for example, where we've determined that a particular product, or category of products, likely will pose less risk of insect resistance developing to a particular PIP protein, we think it appropriate to grant that particular product, or category of products, a registration for a period greater than that granted a corresponding product that poses a greater risk of insect resistance developing. This approach is reflective of complementary principles: first, to ensure that we apply our limited resources to the products that pose greater risk of adverse effects to the environment; and, second, to conserve the resources that registrants and applicants must expend in amending the registrations of products that pose less risk of adverse effects to the environment.

The scheme that we are following includes registration periods of five, eight, and twelve years; a fifteen-year registration period will also be available, if adequately supported by our science assessment. In this scheme, (i) a product with a single PIP toxin, and a 20% external refuge, qualifies for a five-year registration; (ii) a product with pyramided PIP toxins (i.e., two or more toxins with distinct, non-cross reacting modes of action), that are non-high dose (the definition for a high dose product remains unchanged), with either a seed blend or external refuge, qualifies for an eight-year registration; (iii) a product with pyramided PIP toxins (i.e., two or more toxins with distinct, non-cross reacting modes of action), that are **high-dose**, with either a seed blend or external refuge, qualifies for a twelve-year registration; (iv) a product with pyramided PIP toxins (i.e., two or more toxins with distinct non-cross reacting modes of actions), with either a seed blend or external refuge, that has been determined by EPA's science assessment to be 150% as durable as the baseline single toxin product with a 20% external refuge, would qualify for a fifteen-year registration. Products determined by EPA's science assessment to be less than 100% as durable as the baseline single toxin product with a 20% external refuge would not qualify for a five-year registration and the registration period for such products will be determined on a case-by-case basis consistent with the level of risk they pose. Similarly, instances where other risk issues may arise, or where novel resistance concerns may be present, would also be determined on a case-by-case basis, as will novel refuge configurations that may present unique durability profiles.

APPENDIX A

GLOSSARY OF ACRONYMS AND ABBREVIATIONS

ABSTC	Agricultural Biotechnology Stewardship Technical Committee
AIBS	American Institute of Biological Sciences
APHIS	Animal and Plant Health Inspection Service (of the United States Department of Agriculture)
AR	Arkansas
ARS	Agricultural Research Service (of the United States Department of Agriculture)
BPPD	Biopesticides and Pollution Prevention Division
BRAD	Biopesticides Registration Action Document
Bt/*B.t.*	*Bacillus thuringiensis*
°C	Celsius (degrees)
C	carbamate
CAP/CAPs	Compliance Assurance Program/Compliance Assurance Programs
CEW	corn earworm
cm^2	square centimeter/square centimeters
CP4 EPSPS	CP4 5-enolpyruvylshikimate-3-phosphate synthase
CPB	Colorado potato beetle
CFR	Code of Federal Regulations
Co	County
CO	Colorado
Codex	Codex Alimentarius Commission
CRW	corn rootworm
DNA	deoxyribonucleic acid
DT_{50}	half-life
DT_{90}	time until 90% decay
dwt	dry tissue weight
EC_{50}	growth inhibition
ECB	European corn borer
ED_{50}	median effective dose (produces desired effect in 50% of population)
EDSP	Endocrine Disruptor Screening Program
EEC	Estimated Environmental Concentration
EFSA	European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency (the Agency)
EPA Reg. No.	Environmental Protection Agency Registration Number
EPA Reg. Nos.	Environmental Protection Agency Registration Numbers
EUPs	experimental use permits
F_{st}	fixation index
FAW	fall armyworm
FDA	Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act
FR	Federal Register
ft	foot/feet

GLOSSARY OF ACRONYMS AND ABBREVIATIONS, CONTINUED

fwt	fresh weight
g	gram/grams
g/cm^3	grams per cubic centimeter
GIPSA	Grain Inspection, Packers and Stockyards Administration (of the United States Department of Agriculture)
GM	genetically modified
HEEC	Highest Estimated Environmental Concentration
HGT	horizontal gene transfer
IA	Iowa
IgG	immunoglobulin G
IL	Illinois
ILSI-CERA	International Life Sciences Institute Research Foundation – Center for Environmental Risk Assessment
IN	Indiana
IRM	insect resistance management
ISBN	International Standard Book Number
kDa	kiloDalton
kg	kilogram/kilograms
L	liter/liters
lb	pound/pounds
lb/ac	pounds per acre
LC_{50}	median lethal concentration. A statistically derived concentration of a substance that can be expected to cause death in 50% of test animals. It is usually expressed as the weight of substance per weight or volume of water, air, or feed (e.g., mg/L, mg/kg, or ppm).
LD_{50}	median lethal dose. A statistically derived single dose that can be expected to cause death in 50% of the test animals when administered by the route indicated (oral, dermal, inhalation). It is expressed as a weight of substance per unit weight of animal (e.g., mg/kg).
LDR	leaf damage
LLC	limited liability company
LOAEC	Lowest Observed Adverse Effect Concentration
LOC	Level of Concern
LOD	limit of detection
LOQ	limit of quantitation
MALDI-TOF	matrix-assisted laser desorption/ionization time-of-flight
MALDI-TOF-MS	matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MCRW	Mexican corn rootworm
m/d	meters per day
MD	Maryland
MEEC	Maximum Expected Environmental Concentration
meq	milliequivalent
µg	microgram/micrograms
$µg/cm^2$	micrograms per square centimeter
µg/g	micrograms per gram
µg/L	micrograms per liter

GLOSSARY OF ACRONYMS AND ABBREVIATIONS, CONTINUED

µg/mL	micrograms per milliliter
mg	milligram/milligrams
mg/kg	milligrams per kilogram
mg/kg bwt	milligrams per kilogram bodyweight
mg/mL	milligrams per milliliter
MHD	maximum hazard dose
mL	milliliter/milliliters
mL/g	milliliters per gram
mM	millimolar
MN	Minnesota
MRID No./MRID Nos.	Master Record Identification Number/Master Record Identification Numbers
MRL/MRLs	maximum residue level/maximum residue levels
N/A	not applicable
NAS	National Academy of Sciences
NCEAS	National Center for Ecological Analysis and Synthesis
NCGA	National Corn Growers Association
NCR-46	Technical committee consisting of research and extension CRW specialists and other cooperators
NCRW	northern corn rootworm
NE	Nebraska
ng	nanograms/nanograms
NIS	Node/Nodal Injury Scale
NOAEL	No Observed Adverse Effect Level
NOEC	No Observable Effect Concentration
NPTII	neomycin phosphotransferase II
OCSPP	Office of Chemical Safety and Pollution Prevention
OECD	Organization for Economic Cooperation and Development
OP	organophosphate
OPP	Office of Pesticide Programs
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
PC Code	Pesticide Chemical Code
PCA	Phased Compliance Approach
PCR	polymerase chain reaction
PIP/PIPs	plant-incorporated protectant/plant-incorporated protectants
PP	phenyl pyrazoles
ppm	parts per million
PVDF	polyvinylidene fluoride
RDR	root damage rating
RKI	Robert Koch Institute
RNA	ribonucleic acid
RQ	risk quotient
SAP	Scientific Advisory Panel (the Panel)
SCRW	southern corn rootworm
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SGF	simulated gastric fluid
SIF	simulated intestinal fluid

GLOSSARY OF ACRONYMS AND ABBREVIATIONS, CONTINUED

SP	synthetic pyrethroid
SSA	sublethal seedling assay
SWCB	southwestern corn borer
T-DNA	transfer deoxyribonucleic acid
UCS	Union of Concerned Scientists
U.S.	United States
USDA	United States Department of Agriculture
WCRW	western corn rootworm
YGCB	YieldGard® Corn Borer (i.e., MON 810)
YGRW	YieldGard® Rootworm (i.e., MON 863)

Table 1. Currently Registered PIPs Expressing Cry3Bb1 Protein.

EPA Registration Number	Registration Name	Company and Address	Active Ingredient(s)	Initial Date of Registration
524-528*	Corn Event MON 863	Monsanto Company 800 North Lindbergh Boulevard St. Louis, MO 63167	• Cry3Bb1	February 24, 2003
524-545*	MON 863 x MON 810	Monsanto Company 800 North Lindbergh Boulevard St. Louis, MO 63167	• Cry3Bb1 • Cry1Ab	October 31, 2003
524-551	MON 88017	Monsanto Company 800 North Lindbergh Boulevard St. Louis, MO 63167	• Cry3Bb1	December 13, 2005
524-552	MON 88017 x MON 810	Monsanto Company 800 North Lindbergh Boulevard St. Louis, MO 63167	• Cry3Bb1 • Cry1Ab	December 13, 2005
524-576	MON 89034 x MON 88017	Monsanto Company 800 North Lindbergh Boulevard St. Louis, MO 63167	• Cry1A.105 • Cry2Ab2 • Cry3Bb1	June 10, 2008
524-581	MON 89034 x TC1507 x MON 88017 x DAS-59122-7 (or SmartStax™)	Monsanto Company 800 North Lindbergh Boulevard St. Louis, MO 63167	• Cry1A.105 • Cry2Ab2 • Cry1F • Cry3Bb1 • Cry34Ab1 • Cry35Ab1	July 20, 2009
524-583	TC1507 x MON 88017 *Note: This registration is for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed only.	Monsanto Company 800 North Lindbergh Boulevard St. Louis, MO 63167	• Cry1F • Cry3Bb1	December 14, 2009
524-586	MON 88017 x DAS-59122-7 *Note: This registration is for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed only.	Monsanto Company 800 North Lindbergh Boulevard St. Louis, MO 63167	• Cry3Bb1 • Cry34Ab1 • Cry35Ab1	December 14, 2009
524-587	MON 89034 x TC1507 x MON 88017 *Note: This registration is for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed only.	Monsanto Company 800 North Lindbergh Boulevard St. Louis, MO 63167	• Cry1A.105 • Cry2Ab2 • Cry1F • Cry3Bb1	October 15, 2009
524-589	MON 89034 x MON 88017 x DAS-59122-7 *Note: This registration is for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed only.	Monsanto Company 800 North Lindbergh Boulevard St. Louis, MO 63167	• Cry1A.105 • Cry2Ab2 • Cry3Bb1 • Cry34Ab1 • Cry35Ab1	December 14, 2009

EPA Registration Number	Registration Name	Company and Address	Active Ingredient(s)	Initial Date of Registration
524-590	TC1507 x MON 88017 x DAS-59122-7 *Note: This registration is for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed only.	Monsanto Company 800 North Lindbergh Boulevard St. Louis, MO 63167	• Cry1F • Cry3Bb1 • Cry34Ab1 • Cry35Ab1	December 14, 2009
68467-7	MON 89034 x TC1507 x MON 88017 x DAS-59122-7 (or SmartStax™)	Mycogen Seeds c/o Dow AgroSciences LLC 9330 Zionsville Road Indianapolis, IN 46268-1054	• Cry1A.105 • Cry2Ab2 • Cry1F • Cry3Bb1 • Cry34Ab1 • Cry35Ab1	July 20, 2009
68467-8	MON 89034 x TC1507 x MON 88017 *Note: This registration is for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed only.	Mycogen Seeds c/o Dow AgroSciences LLC 9330 Zionsville Road Indianapolis, IN 46268-1054	• Cry1A.105 • Cry2Ab2 • Cry1F • Cry3Bb1	October 15, 2009
68467-10	MON 89034 x MON 88017 x DAS-59122-7 *Note: This registration is for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed only.	Mycogen Seeds c/o Dow AgroSciences LLC 9330 Zionsville Road Indianapolis, IN 46268-1054	• Cry1A.105 • Cry2Ab2 • Cry3Bb1 • Cry34Ab1 • Cry35Ab1	December 14, 2009
68467-13	MON 88017 x DAS-59122-7 *Note: This registration is for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed only.	Mycogen Seeds c/o Dow AgroSciences LLC 9330 Zionsville Road Indianapolis, IN 46268-1054	• Cry3Bb1 • Cry34Ab1 • Cry35Ab1	December 14, 2009
68467-14	TC1507 x MON 88017 *Note: This registration is for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed only.	Mycogen Seeds c/o Dow AgroSciences LLC 9330 Zionsville Road Indianapolis, IN 46268-1054	• Cry1F • Cry3Bb1	December 14, 2009
68467-15	TC1507 x MON 88017 x DAS-59122-7 *Note: This registration is for breeding purposes, agronomic testing, increasing inbred seed stocks, and producing hybrid seed only.	Mycogen Seeds c/o Dow AgroSciences LLC 9330 Zionsville Road Indianapolis, IN 46268-1054	• Cry1F • Cry3Bb1 • Cry34Ab1 • Cry35Ab1	December 14, 2009

*Registration expired on its own terms on September 30, 2010.