BIOPESTICIDES REGISTRATION ACTION DOCUMENT

Event MON863 Bacillus thuringiensis Cry3Bb1 Corn

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

Table of Contents

I. Overview I1

A. Executive Summary

B. Use Profile

C. Regulatory History

II. Science Assessment IIA1

A. Product Characterization IIA1

B. Human Health Assessment IIB1

C. Environmental Assessment IIC1

D. Insect Resistance Management IID1

E. Benefits IIE1

III. Terms and Conditions of the Registration III1

IV. Regulatory Position for Event MON863 Cry3Bb1 Corn IV1

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I. Overview

A. Executive Summary

EPA has conditionally registered the Monsanto Company's new active ingredient, *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIR13L) in Event MON863 corn. The Agency has determined that the use of this pesticide is in the public interest and that it will not cause any unreasonable adverse effects on the environment during the time of conditional registration.

At 80 million planted acres, corn is the largest crop grown in the U.S. and accounts for 20% of total agricultural cropland. Over the past 5 years, conventional insecticides have been applied to between 14 to 18 million acres of corn to control CRW. This single corn pest accounts for 1 out of every 7 insecticide applications to agricultural crops. Infested acreage is increasing due to extended diapause and behavior modification as CRW lays it's eggs in soybean fields which are planted in rotational corn. The acres infested with CRW is expected to grow 2.6% per year and, by 2013, to total 39 million acres.

In assessing the potential benefits from MON 863, EPA compared the efficacy of MON 863 to other chemical controls for CRW, evaluated the human health and environmental benefits compared to registered alternatives, estimated the grower benefits, and estimated the chemical pesticide use reduction from adoption of MON 863. EPA made a determination that the registration of MON 863 was in the public interest and that the benefits outweigh the risks.

Both the MON863 registration and the Cry3Bb1 tolerance exemption under 40 CFR Part 180.1214 are set to expire on May 1, 2004. However, Monsanto has indicated that it will request that the tolerance exemption be amended to remove the expiration date. If (1) Monsanto requests such an amendment to the Cry 3Bb1 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 likely will support an extension of the conditional registration for three years beyond the date of registration.

<u>Product Characterization</u>

Event MON863 corn was produced by transforming corn tissue via a method employing bombardment of particles coated with DNA (Vector ZMIR13L) which contained both the *cry3Bb1* and *nptII* genes. The *cry3Bb1* and *nptII* genes were stably introduced into the corn genome as one intact copy. 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 (above ground whole plant). Monsanto is being required to submit expression data in terms of dry weight, as the amount of protein present in the given tissue.

Cry3Bb1 protein is a delta-endotoxin from *Bacillus thuringiensis* spp. *kumamotoensis* and has activity against certain beetles. The wild-type *cry3Bb1* gene was modified to enhance the protein's activity against the corn root worm complex. *The nptII* gene encodes neomycyin phosphotransferase II and was used as a marker protein to identify the corn tissue that had been transformed with ZMIR13L.

Adequate information was submitted to show that the Cry3Bb1 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 used to obtain sufficient material for testing.

Human Health Assessment

Data demonstrate a lack of mammalian toxicity at high levels of exposure (well above exposure levels that are reasonably anticipated in corn) to the pure Cry3Bb1 proteins. Gross necropsies performed at the end of the three acute oral toxicity studies in mice indicated no findings of toxicity attributed to exposure to the test substance. LD_{50} s were greater than 2,700; 2,980; and 3,780 mg Cry3Bb1 protein/kg body weight (highest doses tested).

The potential for the Cry3Bb1 proteins to be food allergens is minimal. Data demonstrate that the Cry3Bb1 protein is rapidly degraded by gastric fluid in vitro. Further, a comparison of amino acid sequences of known allergens and toxins uncovered no evidence of any homology with Cry3Bb1.

A tolerance exemption exists under 40 CFR 180.1214 (for Bacillus thuringiensis Cry3Bb1 protein and the genetic material necessary for its production in corn) that includes Event MON863. This tolerance exemption expires on May 1, 2004. Monsanto has indicated it will submit a petition in the near future to amend the tolerance exemption so that the expiration date is removed.

The extraction and detection method submitted in support of 40 CFR 180.1214 is adequate for analysis of Cry3Bb1 protein in corn grain. However, Monsanto is required to submit method valdiation data by an independent laboratory, as well as reagents to the EPA's Office of Pesticide Programs Ft. Meade Laboratory for their validation of the method. In addition, to assure that grain handlers have a test method in place prior to harvest, Monsanto must make available Cry3Bb1 strip tests to grain handlers. EPA further understands that these are 'qualitative' test kits and that Monsanto is in discussions with USDA/GIPSA about providing methodology and reagents for their use in developing a validated 'quantitative' method for MON 863.

Environmental Assessment

The Agency has 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 proteins 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 Scientific Advisory Panel (SAP) recommendations, consultations with scientific experts, and public comments received by the Agency.

The Agency assesses the toxicity of Cry3Bb1 protein to representatives of potentially exposed non-target organisms by a tiered testing system using single species maximum hazard dose laboratory data using mortality as the end point. The toxicity of the Cry3Bb1 protein has been evaluated following challenge of several species of invertebrates including: adult and larval honey bees, a parasitic hymenopteran (*Nasonia*), green lacewings, lady beetles, collembola, monarch butterfly, and earthworms. Reproductive and developmental observations were also made on collembola, honeybee and lady beetle larva maturation studies. 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. Although Bt Cry proteins are very specific in their activity to only certain insect species, the Agency has examined the potential toxicity of Cry3Bb1 protein to birds, fish, and mammals. After evaluating all of the data, the SAP report, and the public comments, the Agency has concluded that no unreasonable adverse effects on non-target organism are expected from Cry3Bb1 protein produced in field corn for the duration of this conditional registration. However, some additional non-target insect studies are needed as confirmatory data and EPA has concluded that it is appropriate for long term environmental effects to be assessed by appropriately designed field monitoring during the initial years of the Cry3Bb1 corn registration.

EPA has determined that the use of Cry3Bb1 in field corn will not cause adverse effects to threatened or endangered species. The Cry3Bb1 protein appears to be specifically toxic to Chrysomelid beetles and currently there are no Chrysomelid species listed on the endangered species list. The habitats of endangered/threatened beetle species do not overlap with corn fields. For endangered aquatic beetles, EPA considered the amount of corn pollen in water. Even using the extremely conservative estimate that 100% of the corn pollen was deposited on the water, the concentration of Cry3Bb1 in pollen was several orders of magnitude below the toxic level for any insect.

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, the 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.

However, 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 (IPM) 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 or territories. The fate of Cry3Bb1 protein in soils and indirect effects on soil biota have 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 a 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, actinomycetes, fungi, protozoa, algae, nematodes, springtails or earthworms. In addition, new plants planted in soil containing Bt Cry protein do not take up the Bt protein.

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. However, after consultation with the FIFRA Scientific Advisory Panel in August, 2002 and from several public comments, the Agency is requesting additional data. The supplementary studies would provide additional weight to support the Agency's conclusions. Refer to section C for additional details on the Agencies assessment and requirement for additional data.

Insect Resistance Management

Corn expressing the Cry3Bb1 protein is intended to provide protection against certain species of the corn rootworm (CRW) including the western corn rootworm (*Diabrotica virgifera virgifera*), northern corn rootworm (*D. barberi*) and Mexican corn rootworm (*D. virgifera zea*). Monsanto acknowledges that a robust and practical IRM plan will require time to develop and they are proposing a three-year interim plan. An interim plan was submitted by Monsanto because they

believe growers need to be able to grow MON 863 corn for a period of time so that important information can be generated and growers can be provided an understanding of corn rootworm IRM requirements.

It should be noted that previous IRM assessments for Plant-Incorporated Protectants (PIPs) needed to consider the potential for resistant organisms feeding upon the PIP affecting the performance of registered microbial Bt pesticides against those organisms. In the case of CRW, there are no registered microbial or PIP products for the control of this organism at this time. Likewise, cross-resistance to Cry proteins in other PIPs or microbial products is not an issue.

Monsanto submitted many documents in support of their proposed IRM plan. An IRM plan for MON 863 corn dated June 20, 2000 was submitted to the Agency. An amended IRM plan dated January 8, 2002 was submitted to the Agency intended to supercede the previous submission. Additional preliminary research dated February 20, 2001 was submitted to the Agency. A 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. In response to the SAP, Monsanto submitted additional information to EPA on December 13, 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 were incorporated into the final review.

A 20% non-Bt corn refuge is sufficient for a 3 year interim period while additional information is being gathered. 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. If a 20% refuge is planted as row strips within a corn field, then the strips must consist of at least 6 to 12 consecutive rows.

Seed and granular insecticide treatments to control CRW larvae are acceptable on refuge acres. However, it is not acceptable 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, 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. Non-Bt refuges should not be planted on first year corn fields if the MON 863 hybrids are planted on rotated fields.

Additional research is needed to establish a long-term IRM strategy for MON 863 corn. The August 2002 SAP recognized areas of research recommended by the NCR 46 and identified ten additional areas needing further investigation for a basic scientific assessment. EPA is requiring studies on pest biology and genetics, if possible, the development of resistant laboratory colonies, evaluation of other IRM options, improved computer models, affect of MON 863 on CRW fitness, and additional information monitoring for resistance and mitigation/remedial action. Details of the research needs are in the IRM chapter and the requirements are listed in the terms and conditions of the registration.

Benefits

In assessing the potential benefits from MON 863, EPA compared the efficacy of MON 863 to other chemical controls for CRW, evaluated the human health and environmental benefits compared to registered alternatives, estimated the grower benefits, and estimated the chemical pesticide use reduction from adoption of MON 863. EPA made a determination that the registration of MON 863 was in the public interest and that the benefits outweigh the risks.

MON 863 corn is as effective or more effective than chemical insecticides in protecting corn roots from CRW larval feeding damage, based on the review of the submitted field efficacy studies. Without MON 863 or conventional insecticides, CRW can reduce yields from up to 9 to 28%. Considering all of the efficacy studies reviewed using MON 863 and non-transgenic corn, MON 863 generally experienced less root damage, often significantly less root damage, from southern, northern, and western corn rootworm than a non-transformed control hybrid even when the non-transformed corn was treated with registered soil insecticides. Less root damage has been shown to be correlated with better yields.

MON 863 corn offers practical advantages to corn growers over the current registered alternatives. It can be planted early for a longer growing season and potentially higher yield, while ensuring adequate CRW protection throughout the growing season. In addition, growers should be able to plant their crop more quickly because they won't have to continually have to 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, MON 863 is labeled for general use and will replace current reliance on restricted use products. MON 863 will provide the grower and other occupational workers greater safety, protect water bodies from run-off, and mitigate spray-drift and potential impacts on local non target organisms, such as bird populations.

Based on the Agency's review of the submitted studies, the 50-year history of safe use of Cry proteins in U.S. agriculture, and comparable results to studies of other proteins in the Cry3 class,

EPA has determined that there is a reasonable certainty of no harm resulting from exposure to this protein. MON 863 corn presents no unreasonable risks to humans during any stage of its life cycle, from production, handling, storage, ingestion, to disposal. Cry3Bb1protein has no toxic effects on mammals, and is not likely to induce allergic or hypersensitive responses based on results in all appropriate tests. MON 863 is less toxic than all of the major insecticides currently used to control CRW damage.

All of the major chemicals used for CRW control can cause adverse environmental effects under conditions of normal use. 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. EPA has identified 10 insecticides used in agriculture as the most toxic to birds and 3 are currently used to control the corn rootworm (carbofuran, phorate and methyl parathion). In contrast, the Cry3Bb1 protein has no toxic effects on non-target organisms based on results in all appropriate tests. In addition, Cry3Bb1 is degraded rapidly in the soil (reducing non-target exposure). The Cry3Bb1 protein is expressed by the corn plant; thus, reducing the exposure to non-target organisms from application spillage. In addition, Cry3Bb1 has a narrow target range (beetles of the family Chrysomelidae). The family Chrysomelidae contains no known endangered species. To date, there have been no functional receptors for Cry proteins on intestinal cells of fish, birds, or mammals. As labeled, MON 863 corn poses less risk to the environment than the registered alternatives.

Cumulative grower benefits are projected to be $49.2 million for the three year period 2003 to 2005. The time necessary to develop corn hybrids containing Cry3Bb1 and the time necessary to obtain full European Union approval are key factors affecting the growth rate in benefits. At full commercial maturity when offerings are available for all infested acreage, annual grower benefits are predicted to be $110 million per year. Grower benefits are defined as the difference between the value of MON 863 and it's cost. The value is based on expected yield improvements, reduced costs for insecticides, and practical benefits related to a more flexible and safer product for growers to use than the alternatives. Average grower benefits are estimated to be $6.56/acre which is about 2% of gross income. Crop budgets suggest a net return per acre for corn (not including land charges) of around $60 per acre so MON 863 has the potential to increase profits by 10%. The benefit estimates include all economic costs, such as out of pocket plus opportunity costs. This includes an estimated MON 863 technology fee as well as costs of market acceptance and refuge requirements unique to genetically-modified organisms (GMO's).

Pesticide use reduction projections indicate that as MON 863 CRW-protected corn adoption increases in the next three years, acre treatments will be reduced extensively for all chemical insecticides used currently to control CRW. The greatest use reductions are seen in both the organophosphate and synthetic pyrethroid classes. By 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 current projections. By 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.

B. Use Profile

- **Pesticide Name:** *Bacillus thuringiensis* Cry3Bb1 Protein and the Genetic Material Necessary for its Production (Vector ZMIR13L) in Event MON863 Corn

- **Trade and Other Names:** Corn Event MON863, YieldGard Rootworm™

- **OPP Chemical Code: 006484**

- **Basic Manufacturer: Monsanto Company**
  700 Chesterfield Parkway North
  St. Louis, MO 63198

- **Type of Pesticide: Plant-Incorporated Protectant**

- **Uses: Field Corn**

- **Target Pest(s): Corn Rootworm.**

C. Regulatory History and Public Comments

EUPs and Tolerance Exemption

Several experimental use permits and amendment/extensions have been granted by the Agency since April 2000 for different Cry3Bb1 corn plant-incorporated protectants, EPA EUP Numbers 524-EUP-90, -92, and -93. A tolerance exemption was established for *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material for its production in corn on May 11, 2001. This tolerance exemption was codified under 40 CFR Part 180.1214 and expires on May 1, 2004. Monsanto has indicated their intent to submit a petition amending the Cry3Bb1 corn tolerance exemption to remove the expiration date once they obtain commercial approval.

Registration Application and Public Comments

On March 1, 2001, EPA announced receipt of Monsanto's Event MON863 Cry3Bb1 corn seed increase registration application (EPA File Symbol 524-LEI) pursuant to the provisions of section 3(c)(4) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (66 FR 15435). On

March 13, 2002, EPA announced receipt of a revised application from Monsanto for EPA File Symbol 524-LEI for full commercial use. Finally, on July 24, 2002, EPA announced a meeting of the FIFRA Scientific Advisory Panel (SAP) to consider and review corn rootworm plant-incorporated protectant insect resistance management and non-target insect issues (67 FR 48461).

Approximately 937 comments were received by EPA in response to these announcements. Comments were received from private citizens, independent farmers, farm cooperatives, farm industry representatives, trade organizations, advocacy groups, academic researchers, and government officials. Comments received included handwritten letters, typed letters, e-mails, faxes, and slide presentations from the SAP meeting.

Several organizations interested in the registration of MON 863 sponsored letter writing campaigns to promote their interests and provided form letters for members or other interested citizens to submit to EPA. Six master form letters were identified. The originating organizations for these campaign letters were not apparent, and the master letters were identified on the basis of the most consistently copied text. Most of the 937 comments received were form letters (81 percent). In some cases the form letters were signed and mailed without alteration. However, many commenters added personalized comments or edited the form letters to better express their individual views.

About 88 percent of the 937 comments were received from private citizens. Support or opposition of the registration was not tracked for individual commenters; however, all six of the master form letters expressed support for the registration of MON 863. Based on the large number of form letters received and the large number of letters received from private citizens, most of the private citizens who commented on the application generally supported the registration.

Some of the major environmental or public interest groups included the Center for Science in Public Interest, Foundation E.A.R.T.H., Greenpeace, The Sierra Club, The Xerces Society, and Union of Concerned Scientists. The Center for Science in Public Interest did not express strong support or opposition to the registration of MON 863, but did provide a detailed analysis identifying perceived flaws in the Insect Resistance Management (IRM) plan. Foundation E.A.R.T.H., an environmental organization focusing on agriculture, supported approval of the registration based on an impact survey of 300 corn growers who, by and large, were interested in reducing pesticide use and using a seed product like MON 863. Greenpeace, The Sierra Club, and The Xerces Society, all environmental advocacy groups, along with the Union of Concerned Scientists, a public interest group, opposed approval of the application claiming that insufficient scientific data are available. Each of these groups provided lengthy comments and/or independent evaluations of the application materials submitted by Monsanto.

Agricultural trade associations generally supported approval of registration of MON 863. Among these trade associations were the American Seed Trade Association, the Agribusiness Association of Iowa, the Illinois Corn Growers Association, the Iowa Corn Growers Association, the Nebraska

Corn Growers Association, the Iowa Seed Association, the National Grain and Feed Association, and the National Wild Turkey Federation. A representative of the National Corn Growers Association supported the registration and expressed the importance of a workable IRM plan. The Illinois Corn Growers Association expressed concerns about handling and marketing the product.

Approximately 20 seed and grain companies submitted comments in support of registration of MON 863. Entomologists from research and academic organizations including the NCR-46 committee (a technical committee of research and extension entomologists), Iowa State University, Kansas State University, Michigan State University, Pennsylvania State University, and the University of Delaware also expressed support of the registration. Government representatives of the Illinois Department of Agriculture, Illinois State Senate, Iowa Department of Agriculture, Nebraska Department of Agriculture, Ohio Department of Agriculture, Texas House of Representatives, Texas Senate, and U.S. Department of Agriculture all supported registration of MON 863.

II. Science Assessment

The classifications that are found for each data submission are assigned by the EPA science reviewer and are an indication of the usefulness of the information contained in the documents and if the data meet the intent of the test guidelines. A rating of "ACCEPTABLE" indicates the study is scientifically valid and has been satisfactorily performed according to accepted EPA guidelines or other justified criteria. A "SUPPLEMENTAL" rating indicates the data provide some information that can be useful for risk assessment. However, the studies may either have certain aspects not determined to be scientifically acceptable (SUPPLEMENTAL. UPGRADABLE) or that the studies have not been done to fulfill a specific EPA guideline requirement. 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 current EPA guidelines or does not need to be reclassified as "ACCEPTABLE." Both ACCEPTABLE and SUPPLEMENTAL studies may be used in the risk assessment process as appropriate.

A. Product Characterization

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, on the actual DNA inserted into the plant, on the inheritance and stability of these traits in the plant, on biochemical characteristics of the *Bt* Cry3Bb1 protein and on *Bt* Cry3Bb1 protein expression levels for various plant tissues.

Transformation system: Cry3Bb1 plant-incorporated protectants were transformed into corn tissue via a method employing bombardment of particles coated with DNA encoding the intended insert.

Each plasmid description includes a reference to the strains of *Bacillus thuringiensis* 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 introduced to improve or restrict 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).

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 which 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 the possibility of 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.

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. MON863 did not show independent assortment of the introduced traits (usually the marker protein and the *Bt* protein were examined). This indicates that the traits were on the same chromosome and closely linked (crossover events between the two traits were not detected).

The submissions that covered characterization of the actual DNA insert and stability/inheritance data are listed in the MRIDs for each product. These submissions are acceptable and fulfill this data requirement.

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 2000 and 5000 mg of protein per 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 (ecological effects testing differs and is addressed in the ecological effects section

of this document). 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: molecular sizing by SDS-PAGE and western blot analysis; immunorecognition using ELISA and western blot analysis; N-terminal amino acid sequencing; 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 ecological effects assessment, these non-target pest tests are also covered in the ecological effects assessment.

The *Bt* protein expression level in various tissues throughout the growing season has been determined. However, the data was presented on a fresh weight basis. Expression data in terms of dry weight leaf, root, pollen, seed, and whole plant should be submitted to complete the database. Data obtained for roots should be at typical times when corn rootworm would be feeding as well as at the end of the season. Leaf, whole plant, seed, and pollen data should be from young plants or about the time of pollen release and at the end of harvest when material may be tilled into the soil.

Residue Analytical Methods

Independent laboratory method validation (under OPPTS Guidelines OPPTS 860.1340) and EPA laboratory method validation are necessary 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. 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.

Cry3Bb1 BACKGROUND

Cry3Bb1 protein is a delta-endotoxin from *Bacillus thuringiensis* spp. *kumamotoensis* and has activity against certain beetles. The wild-type *cry3Bb1* gene was modified to enhance the protein's activity against the corn root worm 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 at 5 and 4 amino acid positions respectively. 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 MON863). At the 5' end of the *cry3Bb1* 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.

Data based on Cry3Bb1.11098 and Cry3Bb1.11231 proteins supported an exemption from the requirement of a food tolerance and a tolerance exemption (40CFR§180.1214) was published May 11, 2001.

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 *cry3Bb1* 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), since this protein is produced by the MON 863 corn line rather than the Cry3Bb1.11098 protein.

The Agency has reviewed the additional data submitted by Monsanto in connection with MON863 and concluded that the data provided supports 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 exemption from the requirement of a food and/or feed tolerance (40CFR§180.1214).

GUIDELINE NO	**STUDY**	**RESULTS**	**MRID NO.**
885.1100	Product Characterization (Transformation System and Inheritence and Stability After Transformation)	MON853, MON860, MON862, and MON863 were produced by the incorporation of one of three constructs [PV-ZMIR12L (MON862), PV-ZMIR13L (MON863) or PV-ZMIR14L (MON853 & MON860)] via a particle bombardment mechanism.. The *cry3Bb1* and *nptII* genes were stably introduced into the corn genomes, as determined by at least three generations of greenhouse and field studies. Acceptable.	448779-01
885.1100	Product Characterization (DNA Characterization)	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 which encodes for both Cry3Bb1 and NPTII proteins. ACCEPTABLE	454240-02 451568-02

GUIDELINE NO	**STUDY**	**RESULTS**	**MRID NO.**
885.1100	Product Characterization (Sequencing & Immunoreactivity)	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. However, these changes 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
885.1100	Product Characterization (Protein Equivalence)	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. ACCEPTABLE	451568-03 454240-05
885.1100	Product Characterization (Protein Equivalence)	This report compares the physical (MW, N-terminal sequencing) and functional (bioassay) characteristics of Cry3Bb1.11098 and Cry3Bb1.11231 proteins produced in *E. coli* and CRW protected corn. The data provided show that the proteins have equivalent molecular weight, immunological reactivities, N-terminal sequences and comparable LC_{50} values. This 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

GUIDELINE NO	**STUDY**	**RESULTS**	**MRID NO.**
885.1100	Product Characterization (Protein Equivalence)	Two genetic variants designated as *cry3Bb1.11098* and *cry3Bb1.11231* produce the δ-endotoxin proteins Cry3Bb1.11098 and Cry3Bb1.11231, respectively. Cry3Bb1.11098 differs from the wild type *B.t.* protein by 5 amino acids, while the Cry3Bb1.11231 protein differs by 4 amino acids. The *cry3Bb1.11098* gene was used to develop maize line MON 863 and variant *cry3Bb1.11231* 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 seven and five 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 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 δ-endotoxin proteins. Classification: Acceptable.	454240-10

GUIDELINE NO	**STUDY**	**RESULTS**	**MRID NO.**
885.1100	Product Characterization (Amino Acid Sequencing)	Transformation event MON 863 (maize) produces the 74 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 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 was obtained successfully. The presumed reason for this rests with the post-translational modifications that are typical of eukaryotes (*e.g.*, plants) which 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.	454240-11

GUIDELINE NO	STUDY	RESULTS	MRID NO.
885.1100	Product Characterization (Protein Equivalence)	MALDI-TOF analysis of the microbial and corn Cry3B1.11098(Q349R) proteins yielded an agreement of from 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 position 2 and 32. In the corn Cry3Bb1, three different starts were detected at position 19, 25 and 36. The immunoblot analysis gave similar positive band patterns that indicated the Cry3Bb1 protein produced in both corn and *E. coli* had essentially the same electrophoretic mobility and immunoreactivity. The positive bands themselves were sometimes rather broad (74-66kDa) 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 twofold 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 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 (table 5 attached) 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

GUIDELINE NO	**STUDY**	**RESULTS**	**MRID NO.**
885.1100	Product Characterization (Protein Levels)	The protein titer data provided for MON860 and MON853 shows 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. However, such a determination cannot be made based upon the data provided in this 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 MON853 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 (above ground whole plant). Ranges of Cry3Bb1 protein levels in MON860 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 (above ground whole plant). ACCEPTABLE	449043-02
885.1100	Product Characterization (Protein Levels)	The protein titer data provided shows 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 (above ground whole plant). CLASSIFICATION: Acceptable.	454240-01 451568-02

GUIDELINE NO	STUDY	RESULTS	MRID NO.
885.1100	Product Characterization (Agronomic Performance)	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 a variety of differences in corn ear height, plant height, weight, grain moisture and yield, but in each case, the difference was small. However, based upon the data provided, 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.	453484-03
860.1340	Validated Method for Extraction and Direct ELISA Analysis of Cry3Bb1in Corn Grain	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. Acceptable, pending validation as described.	453731-01

B. Human Health Assessment

1. Background

The basic premise relied on for the toxicology assessment is the fact that all the *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 is often used such as the *Bacillus thuringiensis* source organism or an industrial fermentation microbe. The justification for employing this alternative source of pure protein is the equivalence data discussed above under product characterization.

EPA believes that protein instability in digestive fluids and the lack of adverse effects using the maximum hazard dose approach in general eliminate the need for longer-term testing of *Bt* protein plant-incorporated protectants. Dosing of these animals with the maximum hazard dose, along with the product characterization data should identify potential toxins and allergens, and provide an effective means to determine the safety of these protein. The adequacy of the current testing requirements was discussed at the June 7, 2000 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.

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 an 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. Heat Stability and Amino Acid Homology

Two additional characteristics that are considered as an indication of possible relation to a food allergen are a protein's ability to withstand heat or the conditions of food processing and its 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, the proteins are not considered toxic.

2. Cry3Bb1 Assessment

Pursuant to section 408(b)(2)(D) of FFDCA, EPA has reviewed the available scientific data and other relevant information in support of this action and considered its validity, completeness, and reliability and the relationship of this information to human risk. EPA has also considered available information concerning the variability of the sensitivities of major identifiable subgroups of consumers, including infants and children.

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 productsat levels well above maximum possible exposure levels that are reasonably anticipated in the crops. This is similar to the Agency position regarding toxicity and the requirement of residue data for the microbial Bacillus thuringiensis products from which this plant-incorporated protectant was derived (See 40 CFR 158.740(b)(2)(i)). 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 the observed effects and clarify the source of these effects (Tiers II and III).

Three acute oral studies were submitted for Cry3Bb1 proteins. 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 performed at the end of the study 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 either study.

When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Sjoblad, Roy D., et al. ``Toxicological Considerations for Protein Components of Biological Pesticide Products," Regulatory Toxicology and Pharmacology 15, 3-9 (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 Cry3Bb1 proteins to known toxic proteins available in public protein data bases.

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

Data have been submitted that demonstrate that the Cry3Bb1 protein is rapidly degraded by gastric fluid in vitro. In a solution of simulated gastric fluid (pH 1.2 - U.S. Pharmacopeia), complete degradation of detectable Cry3Bb1 protein occurred within 30 seconds. Insect bioassay data indicated that the protein loss insecticidal activity within 2 minutes of incubation in SGF. Incubation in simulated intestinal fluid resulted in a~59 kDa protein digestion product. 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.

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, Roy D., et al. ``Toxicological Considerations for Protein Components of Biological Pesticide Products," Regulatory Toxicology and Pharmacology 15, 3-9 (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.

Aggregate Exposures

Pursuant to FFDCA section 408(b)(2)(D)(vi), EPA considers available information concerning aggregate 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 protectant chemical residue, and exposure from non-occupational sources. Exposure via the skin or inhalation is not likely since the plant-incorporated protectant is 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 toxicity demonstrated for the Cry3Bb1 proteins.

Cumulative Effects

Pursuant to FFDCA section 408(b)(2)(D)(v), 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 to these plant-incorporated protectants, we conclude that there are no cumulative effects for the Cry3Bb1 proteins.

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

A. Toxicity and Allergenicity Conclusions

The data submitted and cited regarding potential health effects for the Cry3Bb1 proteins include the characterization of the expressed Cry3Bb1 protein 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 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 acute oral toxicity data submitted supports 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, Roy D., et al. ``Toxicological Considerations for Protein Components

of Biological Pesticide Products," Regulatory Toxicology and Pharmacology 15, 3-9 (1992)). Since no effects were shown to be caused by Cry3Bb1, even at relatively high dose levels (3,780 mg Cry3Bb1/kg bwt), the Cry3Bb1 proteins are not considered toxic. This is similar to the Agency position regarding toxicity and the requirement of residue data for the microbial Bacillus thuringiensis products from which this plant-incorporated protectant was derived. See 40 CFR 158.740(b)(2)(i). 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 the observed effects and clarify the source of these effects (Tiers II and III).

Cry3Bb1 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.

Both available information concerning the dietary consumption patterns of consumers (and major identifiable subgroups of consumers including infants and children); and safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food additives, are generally recognized as appropriate for the use of animal experimentation data were not evaluated. The lack of mammalian toxicity at high levels of exposure to the Cry3Bb1 proteins 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 protectants active ingredients are the nucleic acids (DNA, RNA) which 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 ingredient, 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.

B. 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, the provision requiring an additional margin of safety does not apply. Further, the provisions of consumption patterns, special susceptibility, and cumulative effects do not apply.

C. 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 above, no toxicity to mammals has been observed for the plant-incorporated protectants.

Other Considerations

A. Endocrine Disruptors

The pesticidal active ingredients are proteins, derived from sources that are not known to exert an influence on the endocrine system. Therefore, the Agency is not requiring information on the endocrine effects of these plant-incorporated protectants at this time.

B. Analytical Method(s)

Validated methods for extraction and direct ELISA analysis of Cry3Bb1 in corn grain have been submitted and found acceptable by the Agency.

C. Codex Maximum Residue Level

No Codex maximum residue levels exists for the plant-incorporated protectantsBacillus thuringiensis Cry3Bb1 protein and the genetic material necessary for its production in corn.

D. Occupational Exposure and Risk Characterization

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. Worker exposure to the Cry protein via seed dust is also expected to be negligible because of the low amount of protein expressed in transformed plants. If such exposure should occur, the Agency concludes that such exposure would not be expected to present any risk due to the lack of toxicity. However, if any unreasonable adverse effects caused by

exposure to Cry3Bb1 are identified, these effects must be reported to the Agency as described in Sec. 6(a)(2) of FIFRA.

E. Current Tolerance Exemption

Sec. 180.1214 Bacillus thuringiensis Cry3Bb1 protein and the genetic material necessary for its production in corn; exemption from the requirement of a tolerance.

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.This exemption from the requirement of a tolerance will expire on May 1, 2004.

Guideline No	Study	Results	MRID
885.3050	Acute Oral Toxicity	There did not appear to be significant adverse affects to animals dosed with Cry3Bb1 at corrected dose amounts of 38.7, 419, or 2980 mg/kg bodyweight. Two animals died during the study - animal #s 98035M3-007 and 98035F3-004 in the 2980 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 LD_{50} for Cry3Bb1.11098 is greater than 2980 mg/kg bodyweight in mice. ACCEPTABLE.	449043-06
885.3050	Acute Oral Toxicity	There were no apparent adverse effects identified in mice dosed orally with 36, 396 and 3780 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 3780 mg/kg bodyweight in mice. ACCEPTABLE.	449043-05

Guideline No	Study	Results	MRID
885.3050	Acute Oral Toxicity	There did not appear to be significant adverse affects to animals resulting from exposure to Cry3Bb1.11098(Q349R) at dose amounts of 300, 900 & 2700 mg/kg body weight. Observations included some minor clinical affects 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 3200 mg/kg body weight in mice. CLASSIFICATION: Acceptable.	455382-02
	In vitro Digestibility	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 loss 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 for at least 30 minutes. ACCEPTABLE. Neither Cry3Bb1.11098 nor Cry3Bb1.11231 appear to be stable to digestion in simulated gastric fluid for more than 30 seconds. However, there appears to be a discrepancy between the results discussion and figures 2 & 4. The Cry3Bb1.11098 protein appeared to be significantly digested within 15 seconds and completely digested in less than 30 seconds based upon the figures provided. This differs somewhat with the discussion provided by the registrant which indicates that the protein was digested to undetectable levels between 30 and 60 seconds. These do represent drastically different digestion times, but the discrepancy should be addressed. This study was conducted in accordance with Good Laboratory Practice guidelines with four exceptions as described in the submission. These exceptions should not have a significant impact on the outcome of the study. CLASSIFICATION: Acceptable.	449043-07

Guideline No	Study	Results	MRID
885.1100	*In vitro* Digestibility	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 loss 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
885.1100	*In vitro* Digestibility	The tests performed in this study show that the Cry3Bb1 proteins are degraded in simulated gastric fluid. Incubation of corn-produced and *E. 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
	In vitro digestibility	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 and 57kDa at the first assay time point of 1 minute. The subsequent samples (from 5 minute to 24 hours) all gave a single 57 kDa band which did not appear to decrease in intensity. This lack of degration by intestinal fluids is similar to the majority of Cry proteins which are resistant to the action of trypsin. CLASSIFICATION: ACCEPTABLE.	455770-02
	Product Characterization (Heat Stability)	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 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

Guideline No	Study	Results	MRID
885.1100	Product Characterization (Heat Stability)	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
885.1100	Product Characterization (Toxin Database Comparison)	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 this 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	449043-08
885.1100	Product Characterization (Allergen Database Comparison)	Several amino acid database comparison tools were employed to compare the amino acid sequence of Cry3Bb 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 this data, it does not appear that Cry3Bb1 shares significant structural, biological or immunological similarity with known protein allergens or gliadins. Classification: ACCEPTABLE	449043-09 454240-08

Guideline No	Study	Results	MRID
	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 δ-endotoxin when compared to the sequence as found in wild type *B. thuringiensis. B.t.* strains EG11231 and EG11098 contain variants of the Cry3Bb1 protein which differ from the wild type δ-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 *B. thuringiensis* cells expressing variant 11231 was used in toxicology studies for environmental and mammalian concerns. Functional and physicochemical equivalence between variant 11231 produced in *B.t.* 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 which exceeded the concentration of Cry3Bb1 toxins expected in the maximum environmental exposure. NOECs 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 *B.t.* δ-endotoxins, their widespread use in agriculture, the rapid digestibility of Cry3B proteins, their lack of homology to known toxins and allergens, and the safety of the microbial biopesticide Raven® , which expresses Cry3B proteins, the Cry3Bb1 protein is expected to have a reasonable certainty of causing no harm in its aggregate exposure.	454240-09