II. Science Assessment

A. PRODUCT CHARACTERIZATION

Product characterization is critical to understanding the way in which the registered products were made and the unique characteristics that need to be assessed for each *Bt* plant-pesticide. 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* protein and on *Bt* protein expression levels for various plant tissues. Specific information and data for each of the registrations seeking renewal are included in tabular and descriptive formats.

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.

The following table summarizes the registered *Bt* protein-containing plant-pesticide products being evaluated.

Table A1 - *Bt* Plant-Pesticide Products

Common Name and Cry Protein	OPP Chemical Code	Company	Plasmid ID	Plant/ Trade Name
*Bt*11 Cry1Ab *Bt* Corn	006444	Novartis	pZO1502	YieldGard, Attribute
MON810 Cry1Ab *Bt* Corn	006430	Monsanto	pvZMCT01* pZMBK07 pZMGT10**	YieldGard
CBH351 Cry9C Corn	006466	Aventis	pRVA9909 pDE110**	StarLink
Cry1Ac *Bt* Cotton	006445	Monsanto	pvGHBK04	BollGard
Cry3A *Bt* Potato	006432	Monsanto	pvSTBT02	NewLeaf

* pvZMCT01 was a mixture of two plasmids
** Plasmid contains marker gene.

Transformation systems: Registered corn products were transformed using protoplast electroporation to introduce the desired DNA or methods employing bombardment of particles coated with DNA encoding the intended insert. *Agrobacterium tumefaciens*-mediated transformation was used for both the cotton and potato products.

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. 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. When the inserted DNA construct includes traits expressed only in bacteria and not expected to be expressed in the plant, data have been presented to indicate that there is no transcription or translation of the bacterial trait (e.g., *ori* and *amp*^{r} - discussed further in the horizontal gene transfer section).

Inheritance and Stability after Transformation: The data generated for this endpoint examines 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. None of the *Bt* plant-pesticide products showed 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. Each of these submissions are acceptable and fulfill this data requirement. It should be noted that stability and inheritance were not addressed with the registrations for MON810 (006430) and Cry3A (006432). However, considering the use of these crops for several growing seasons and the lack of reports relating to loss of efficacy due to *Bt* protein expression, this specific endpoint can be considered to have been addressed through commercial use.

Protein Characterization and Expression: For the *Bt* plant-pesticides, 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. These equivalency data were generated for all products registered to date.

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; immunorecogniton using ELISA and western blot analysis; N-terminal amino acid sequencing; 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 for each product. As this is a major aspect of the high-dose strategy determination for insect resistance management, the impact of protein expression levels are also covered in that section of the *Bt* crops reevaluation. The nominal protein expression levels as determined by field and/or greenhouse conditions are described below. Note that there may be variation between the *Bt* protein values reported by each company due to differences in the antibody-based reagents used for quantifying the *Bt* protein. There are also differences due to reporting *Bt* protein values based on tissue fresh weight. While these differences may make direct comparisons between the tissue expression levels reported by different companies difficult, the reported levels provide enough information to be used for risk assessment purposes especially when considered along with the reported tissue bioactivity values.

Table A2 Cry Protein Tissue Expression

Active Ingredient	Leaf	Root	Pollen	Pith	Seed	Whole Plant
Cry1Ab-*Bt*11 (006444)	3.3 ng/mg	2.2-37.0 ng/mg protein	< 90 ng Cry1Ab/ g dry wt. of pollen	_	1.4 ng/mg (kernel)	_
Cry1Ab-MON810 (006430)*	10.34 microgram/g	_	< 90 ng Cry1Ab/ g dry wt. of pollen	_	0.19-0.39 microgram/g (grain)	4.65 microgram/g
Cry9C (006466)	44 microgram/g	25.87 microgram/g	0.24 microgram/g	2.8 microgram/g (stalk)	18.6 microgram/g (kernel)	250 microgram/g
Cry1Ac (006445)	2.04 microgram/g	_	11.5 ng/g	_	1.62 microgram/g	_
Cry3A (006432)	28.27 microgram/g	0.39 microgram/g (tuber)	_	_	_	3.3 microgram/g

* 1994 Field Data
** All values reflect fresh tissue weight unless otherwise noted.

1. Product Characterization of Bt 11 Cry1Ab Corn (006444)

The corn line Bt 11 was produced by transforming another proprietary corn line with plasmid PZO1502 which contained *cry1Ab, pat* and *amp*^{r} genes. The *amp*^{r} gene was not found in the plant. The *cry1Ab* gene was also altered to improve its GC ratio for expression in corn and coded for a truncated form of the original protein. Both field corn and sweet corn containing the plant-pesticide descend from the original Bt 11 transformant.

Data showed that the truncated Cry1Ab toxin could be extracted from corn leaf tissue and this purified material displays characteristics and activities similar to that produced in *E. coli* which has been transformed to produce Cry1Ab. The purified tryptic core proteins from both plant and microbe were shown to be similar in molecular weight by SDS-PAGE, immunorecogniton in western blots and ELISA, partial amino acid sequence analysis, lack of glycosylation and bioactivity against either European corn borer or corn earworm. This analysis justified the use of the microbially produced toxin as an analogue for the plant produced protein in mammalian toxicity testing.

The product characterization data supporting the registration of *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production (plasmid vector pZO1502) in corn is listed below.

Study Type	Result	MRID #
Transformation System	Corn line HE89 was transformed with plasmid *pZO1502* which contains genes for a truncated Cry1Ab, PAT and AMP^r. The *cry1Ab* gene was also altered to improve its GC ratio for expression in corn. (See MRID No. 437548-01 below which indicates the absence of the *amp^r* gene in the Bt11 and control plants.) CLASSIFICATION: ACCEPTABLE	431308-01
Inheritance and Stability after Transformation	The linkages of the *pat* and *cry1Ab* genes were shown by examining the progeny of two selfed generations derived from a population of corn plants segregating for the desired traits. None of the 2320 plants examined showed the two traits independently assorting which indicates that the loci are tightly linked. CLASSIFICATION: ACCEPTABLE	433526-02
Transformation System	The lack of any positive probe recognition for the plant genomic DNA samples indicate the absence of the *amp^r* gene in the Bt11 and control plants. The positive *amp^r* gene probe results for the plasmid DNA digest samples confirm that a fragment of a size consistent with the 7.2 Kb pZO1502 plasmid contained the *amp^r* gene. This would also be appropriate for any digest which had a single restriction cut site as these enzymes did according to the pZO1502 map. The probe results also indicate that a Not I digest would release the *amp^r* gene from the pZO1502 plasmid CLASSIFICATION: ACCEPTABLE	437548-01
Protein Characterization and Expression	Data is presented showing that the truncated Cry1Ab toxin can be extracted from corn leaf tissue and this purified material displays characters and activities similar to that produced in *E. coli*. The similarities are shown in molecular weight after SDS-PAGE, immunorecogniton in western blots and ELISA of trypsin resistant core proteins, partial amino acid sequence analysis, lack of glycosylation and bioactivity against either European corn borer of corn earworm. CLASSIFICATION: ACCEPTABLE	433972-02

2. Product Characterization of MON810 Cry1Ab Corn (006430)

Monsanto's corn line MON 810 was produced by ballistically transforming another proprietary corn line with plasmid construct PV-ZMCT01. Plasmid construct PV-ZMCT01 consists of plasmids PV-ZMBK07 & PV-ZMGT10 ballistically introduced together. The MON 810 line of corn is similar to MON 801 corn in that they both were derived from transformation events utilizing PV-ZMCT01. The MON 810 only expresses a truncated version of Cry1Ab delta-endotoxin. MON801 expresses the full length version of Cry1Ab and the marker gene products.

MON 810 and MON 801 were each transformed with the same plasmid construct (PV-ZMCT01). The MON 810 progeny express a slightly truncated version of Cry1Ab compared to MON 801, but the active site is still retained. The MON 810 progeny do not express detectable levels of the marker gene products found in MON 801 progeny. Some of the data used to evaluate MON810 corn was generated from MON801 corn. To justify this bridging of data from one corn transformation event to another, the company provided product characterization data to demonstrate the similarities and differences between the two transformation events.

Study Type	Result	MRID #
Transformation System Characterization of the DNA Inserted in the Plant Protein Characterization and Expression	The digests of genomic DNA from corn line MON 80100 revealed that the two plasmids PV-ZMBK07 and PV-ZMGT10 had been inserted apparently at two locations. Full length copies of the cry1Ab, gox, nptII and cp4 epsps genes were found. Less than full length copies of all these genes were also found. Western blot analysis revealed that only Cry1Ab and CP4 EPSPS proteins were expressed at detectable levels in the corn plant. CLASSIFICATION: ACCEPTABLE	435332-01
Protein Characterization and Expression	The antiserum reactions revealed many western blot bands in both the Dipel® and the ECB resistant corn extracts not treated with trypsin. No bands clearly related to the Cry1Ab toxin were seen in the non-transformed plant extracts whereas a band comigrating with the full length Cry1Ac standard (similar in size to Cry1Ab) was seen in both Dipel® and ECB resistant corn. The tryptic digests of Dipel® and ECB resistant corn extracts revealed intensified bands that comigrated with the Cry1Ab tryptic core standard. Together these data infer that the same Cry1Ab protein is being produced in ECB resistant corn plants as is found in the microbial product. CLASSIFICATION: ACCEPTABLE	435332-03
Characterization of the DNA Inserted in the Plant	The Southern blots with the two transforming plasmids PV-ZMBK07 and PV-ZMGT10 indicate that only a portion of the PV-ZMBK07 plasmid was successfully integrated. Western blots indicate that all the constructs tested (MON801, 802, 805, 809, 810, 813 and 814) produce delta endotoxin detectable as tryptic core with anti-Cry1Ac antiserum. The genes of the second plasmid used to transform the corn lines, PV-ZMGT10, which include *CP4 EPSPS* and *gox*, were not detected by Southern blot analysis using the PV-ZMGT10 plasmid as probe. These genes which confer glyphosate tolerance were apparently lost during development of the MON810 line since they had to be present for the original callus culture selection process but were not found in the final line described here. CLASSIFICATION: ACCEPTABLE	436655-01

Study Type	Result	MRID #
Protein Characterization and Expression	The results of the western blot showed the trypsinized extracts of corn lines MON 802, 805, 809, 810, 813, and 814 expressed proteins that comigrated with the Cry1Ab protein as found in MON 801 and the same Cry1Ab protein purified from *E. coli.* These bands also reacted with antiserum #B6 specific for the tryptic core protein of Cry1Ab. These results indicate the trypsinized proteins found in all these plants were of same molecular size (63 kD) and immunoreactivity with the reference standards of Cry1Ab expressed in *E. coli* and corn line MON801. CLASSIFICATION: ACCEPTABLE	436655-03
Protein Characterization and Expression	The Cry1Ab protein produced in *E. coli* was shown by SDS-PAGE, western blot, N-terminal amino acid sequencing, glycosylation and bioactivity to be substantially equivalent to the plant produced Cry1Ab. The test results showed the tryptic core of the plant and microbial protein were of essentially identical SDS-PAGE mobility, immunoreactivity in western blot analysis and N-terminal amino acid sequence for the first 15 positions. A comparison of the dose response relationship of plant and microbial extracts against *Heliothis virescens* and *Helicoverpa zea* indicates that the tested proteins are of similar bioactivity. CLASSIFICATION: ACCEPTABLE. These results allow the substitution of the microbially produced Cry1Ab protein for the plant source in toxicology testing.	435332-04

3. Product Characterization of CBH351 Cry9C Corn (006466)

The Cry9C gene was originally isolated from a *Bacillus thuringiensis* subsp. *tolworthi* strain. The gene was then modified for expression in plants before it was stably inserted into corn plants to produce a truncated and modified Cry9C protein. The tryptic core of the microbially-produced Cry9C protein is similar to the Cry9C protein found in event CBH351 corn, save for a single amino acid substitution in the internal sequence and the addition of two amino acids to the N-terminus. The Cry9C protein was produced and purified from a bacterial host to utilize in the mammalian toxicity studies due to the bacterium's greater production potential. Product analysis that compared the Cry9C protein from the two sources included: SDS-PAGE, western blots, – terminal amino acid sequencing, glycosylation tests (for possible post- translational modifications) and insect bioassays. Results from the spike and recovery tests used to validate the ELISA tests for quantifying Cry9C protein indicated that the ELISA could detect only 60% to 80% of the amount added to a tissue sample. None of the Cry9C protein amounts determined by ELISA in the other studies were corrected for this potential error. There were three different methods for determination of protein concentration done on the purified Cry9C protein standard which gave, not unexpectedly, conflicting results. The dosing amount of Cry9C stated in EPA reviews for the acute oral study was corrected from those given by the company to reflect the results of the most conservative protein concentration determination of Cry9C present.

Study Type	Result	*MRID #*
Grain Composition	Compositional analysis of grain from corn expressing Cry9C and other corn lines sharing similar parental lines indicates the Cry9C corn does not differ from the closely related -*Bt* corn, the standard hybrid or the reference values published by USDA-HNIS. While there were differences from the reference values for protein, ash and several amino acids, there were no changes among the three groups analyzed that would distinguish the +*Bt* hybrid as being nutritionally different from the others. CLASSIFICATION: SUPPLEMENTAL. This analysis is not necessary for risk assessment of the pesticidal substance itself. The results may be appropriate for detecting nutritional changes.	442581-04
Protein Characterization and Expression	The control plant extracts had protein concentrations from 9.0 mg to 21.0 mg/gram plant powder or pollen and no detectable levels of either Cry9C or PAT protein (ELISA). The CBH-351 event corn plant powders had protein levels from 13.5 mg to 24.8 mg/gram plant powder or pollen. The ELISA concentration of Cry9C protein in the powders were 290 microgram (TPP-351-0396) or 359 microgram (TPP-351-0196) per gram plant powder. The Cry9C level in pollen was 0.24 microgram/gram pollen. A European corn borer bioassay of the plant powder gave an LC_{50} value of 31.3 ng/cm^2 compared to 72.9 ng/ml for the purified bacterial Cry9C protein. The Cry9C protein appears to be stable in lyophilized plant powder for at least three months (441µg in TPP-351-0196). CLASSIFICATION: ACCEPTABLE. Note: An "interfering substance" in the Cry9C purified bacterial powder was theorized to account for discrepancies between the Bradford, ELISA and OD_{280} protein assays. It is unclear how this interference was resolved to use the bacterial Cry9C as a standard for the ELISA assay or in the spike and recovery experiment (MRID 443844-02).	442581-05
Insect Host Range Comparison of Cry9C Protein.	This non-guideline study is classified supplementary; it was conducted following GLP regulations 40 CFR 160. The study compared endotoxin activity of Cry9C from three sources (bacteria, corn plant tissue, and corn pollen) by determining the relative sensitivities of larvae of four species of insects. European corn borer, tobacco budworm, and diamondback moth were sensitive to Cry9C, but corn earworm was not sensitive to Cry9C. CLASSIFICATION: SUPPLEMENTAL. No additional information is necessary.	442581-06

Study Type	Result	*MRID #*
Protein Characterization and Expression	Cry9C protein from corn, *Bacillus thuringiensis* and *Escherichia coli* was compared for similarity in activity against European corn borer (ECB) and biochemical characters such as molecular weight, immunoreactivity, N-terminal amino acid sequence and post-translational glycosylation. SDS-PAGE indicated a molecular weight of 67.92kD, 70.47kD and 73.79kD for the *E. coli*, *B. thuringiensis* and corn produced extracts, respectively. Each of the samples has a prominent immunoreactive band at approximately 70kD and other very faint, lower molecular weight bands. The results of the N-terminal amino acid analysis indicate that there is N-terminus protease clipping of from 3 to 7 amino acid residues, possibly during the purification process. The residues obtained in the sequencing results agree with the expected sequence. Staining for the carbohydrates did not reveal any detectable Cry9C protein glycosylation by the data presented although the company claims there is some minor positive staining. The bioactivity against ECB of Cry9C protein from plant or *B. thuringiensis* in a diet surface contamination assay was similar although the values and stability differed. CLASSIFICATION: ACCEPTABLE.	443844-01
Protein Characterization and Expression	The results of testing the three types of extracts indicate the ELISA assay displayed a response over the range of 50 to 250 microgram/ml Cry9C protein that could be plotted as linear (r^2=>0.95) but appears in most cases to be a typical shallow sigmoid curve forced through the origin. The assay showed a similar response function regardless of the extract tested. The percent recovery for two quality control samples of 78.6 and 183.4 microgram/ml ranged from 61.5% and 87.5% in the extraction buffer alone to 59% and 73% in plant protein matrix to 58% and 79% in the total plant powder, respectively. These results indicate a minor interference with the plant protein matrices and a potential recovery of 60% to nearly 80% of the Cry9C protein present. CLASSIFICATION: ACCEPTABLE. We note that these results were not used to correct Cry9C protein values given in other assays. See the Ecological Exposure and Risk Characterization section.	443844-02
Transformation System Characterization of the DNA Inserted in the Plant	Southern analysis of the DNA from event CBH-351 corn indicates that both the *cry9c* and *bar* genes are incorporated into the genome. The *cry9c* gene is found as a single copy whereas the *bar* gene is found in multiple copies (apparently four). Northern analysis of the mRNA indicate that Cry9C is expressed in leaf, stem, root, tassel, and ear. Concentrations of the mRNA present indicate there is a variability in the tissues sampled. There is no indication about the possible variability of Cry9C expression with different developmental stages in the plant. It is unclear if the mRNA levels correlate to Cry9C protein expression levels. Northern analysis indicate that no mRNA from the *bla* gene is detectable as would be expected from a gene under control of a bacterial promoter. CLASSIFICATION: ACCEPTABLE.	442581-01

Study Type	Result	*MRID #*
Characterization of the DNA Inserted in the Plant	Tissue samples included in these new Northern blots include mRNA from wild-type, non-transformed corn (B73), root tissue in two CBH351 plants and dry seeds (lot 96ZM001879). The *cry9c* and *bar* Northern blots both suggest that mRNA for those traits can be found in leaf, root and stem tissue but is not detectable in the dry seeds or control non-transformed plants. These blots indicate no mRNA from the traits found in CBH-351 plants detectable in the B73 control samples. The blots also indicate that plant to plant variation in mRNA levels may not be as great as suggested by the first submission (MRID 442581-01) where the number of plants actually sampled was not mentioned. That no detectable mRNA can be found in dry seeds is expected when examining a metabolically quiescent tissue like mature seed. Without an indication how mRNA levels relate to protein expression levels, these results do not significantly alter the evaluation from the previous review. Additional information relates to the stability of the introduced traits by Southern blot analysis. The Southern blots show essentially identical banding patterns from the *Hind* III digests of genomic DNA from CBH-351 corn probed with pRVA9906. None of the designated B73 wild type corn showed any reactivity with the pRVA9906 probe indicating an absence of any traits from that plasmid. The DNA samples analyzed include a series stated as being derived from different genetic backgrounds. Another set of DNA samples was derived from five generations of traditional crosses. The nomenclature used to designate the plant progeny samples was not apparent from the figure legends. CLASSIFICATION: ACCEPTABLE	443844-03

4. Product Characterization of Cry1Ac Cotton (006445)

Monsanto submitted information which adequately described the Cry1Ac delta-endotoxin from *Bacillus thuringiensis* subsp. *kurstaki*, as expressed in cotton, along with the genetic material necessary for its production. Because it would be difficult, or impossible, to extract sufficient biologically-active toxin from the plants to perform toxicology tests, Monsanto used delta-endotoxin produced in bacteria. Product analysis data was submitted to show that the microbially expressed and purified Cry1Ac delta-endotoxin is sufficiently similar to that expressed in the plant to be used for mammalian toxicological purposes. Plant and microbially produced Cry1Ac delta-endotoxin were shown by these studies to have similar molecular weights and immunoreactivity (SDS-PAGE and western blots), to lack detectable post-translational modification (glycosylation tests), to have identical amino acid sequences in the N-terminal region and to have similar results in bioassays against *Heliothis virescens* and *Helicoverpa zea*. While it is difficult to prove that two proteins are identical, the combined results of the above studies indicate a high probability that these two sources produce proteins that are essentially identical by available protein analytical assays.

Study Type	Result	MRID #
Protein Characterization and Expression Characterization of the DNA Inserted in the Plant	Southern blot analysis on restriction digests of DNA extract from cotton line 531 and the parental Coker 312 showed that there is probably only one insert of the *cry1Ac* gene cassette present in the transformed line. The introduced gene appears to be genetically stable in cotton according to the results of progeny selfing and backcrosses with elite lines. The amino acid sequence is homologous to the *cryIAb* gene from HD-1 for positions 1-466 and homologous to *cry1Ac* for positions 467-1178 with a single exception of a leucine-serine 766 in the crystal portion of the protein cleaved prior to toxin activation. Western blot analysis of purified toxin, leaf tissue from cotton line 531 and the parental Coker 312 shows that trypsinized extracts have comigrating bands similar to that found in *B.t.k.* HD-73 protein reference material and commercial preparations. CLASSIFICATION: ACCEPTABLE.	431452-01
Protein Characterization and Expression	*B.t.k.* HD-73 toxin isolated from either cotton line 531 or 931 were compared to the toxin expressed in *Escherichia coli* (*E. coli*) by SDS-PAGE, western blot, glycosylation and bioactivity. The data presented suggests the bacterially produced protein and that found in cotton are equivalent and suggests the bacterially produced *B.t.k.* HD-73 toxin can serve as a surrogate test substance in the toxicological tests to support the registration of transgenic cotton. The original data package for this study did not have a section describing the purification method to obtain the plant standard, and was classified as supplementary on that basis. Additional information on the purification method as described in "Assessment of Equivalence Between *E. coli*-produced and cotton-produced *B.t.k* HD-73 Protein..." MRID 43152-02 were provided. The additional information was sufficient to clarify the extraction procedure and the study is now ACCEPTABLE.	431452-02
Protein Characterization and Expression	The delta-endotoxin from *B.t.k.* HD-73 (lot # 5025385) produced in *E. coli* containing plasmid (pMON10569) was purified, lyophilized and found to have the following characteristics: 4.5% moisture, 75.6% protein (amino acid analysis), 70% protein (BCA), 88% HD-73 specific protein (ELISA), 80% HD-73 specific protein (coomassie blue PAGE), 1.6micrograms gram-negative endotoxin/mg and no significant trace metals except for sodium, potassium and phosphate. The molecular weight of the *B.t.k.* HD-73 toxin was estimated to be 134.8 kD for the full length species and 77.1 kD for the tryptic fragment. The functional activity was found to be an LC_{50} of 0.28 ppm against *Heliothis virescens*. CLASSIFICATION: ACCEPTABLE.	431452-03

Study Type	Result	MRID #
Protein Characterization and Expression	Ten insect pest species from 5 families were tested for their sensitivity to *B.t.k.* HD-73 protein. Only in the lepidopteran species was there significant mortality. In one study, the green peach aphid showed marginal effects from treatment with a tryptic digest of the Cry1Ac toxin from *B.t.k.* HD-73 which was not reproducible in a repeat test. The tryptic digest preparation positive control from a *B.t.k* species also showed higher mortality in the tobacco budworm test than that produced in *E. coli.* CLASSIFICATION: ACCEPTABLE.	431452-04

5. Product Characterization of Cry3A Potato (006432)

Monsanto submitted information which adequately described the plant-pesticidal substance, *Bacillus thuringiensis* subsp. *tenebrionis* Cry3A delta endotoxin as produced in potato. Because it would be difficult, or impossible, to extract sufficient biologically-active toxin from the plants to perform toxicology tests, Monsanto used an endotoxin produced in bacteria. Product analysis data were submitted to show that the microbially expressed and purified *Bt* Cry3A delta endotoxin is sufficiently similar to that expressed in the plant to be used for mammalian toxicological purposes.

Study Type	Result	MRID #
Characterization of the DNA Inserted in the Plant	The relative size and number of copies of the DNA inserted into potatoes was demonstrated with endonuclease digested chromosomal DNA from field grown potato plants Southern blotted with the introduced plasmid as the probe. These Southern blots provided information about the number of copies of introduced DNA, the lack of significant amount of DNA introduced outside the border regions and integrity of the introduced DNA near the endonuclease cut site. These results indicate that only the DNA necessary to produce the Cry3A delta endotoxin were introduced into the plant. CLASSIFICATION: ACCEPTABLE	429322-01
Protein Characterization and Expression	Microbially-produced delta endotoxin from the *cry3A* gene as expressed in Escherichia coli and in potato tubers was compared. The data consist of SDS-PAGE co-migration, western blot analysis, staining for carbohydrate residues, N-terminal amino acid sequence analysis and biological equivalence against *Leptinotarsa decemlineata.* These data are adequate to support the equivalence of the microbially- and plant-produced protein for use in the toxicology studies. CLASSIFICATION: ACCEPTABLE	429322-02
Protein Characterization and Expression	The purity and activity of a 55kD protein released with tryptic digestion of the *Bt* Cry3A delta endotoxin purified from *E. coli* was shown to have a similar size, immunoreactivity and amino acid sequence to the 55kD fragment found in potato tubers. The 55kD protein had somewhat higher bioactivity than the 68kD full-length delta endotoxin from *B.t.t.* These data support the contention that both the 55kD and 68kD forms of the Cry3A delta endotoxin found in the plant were similar to those occurring in *B.t.t.* CLASSIFICATION: ACCEPTABLE	429322-05

Study Type	Result	MRID #
Characterization of *E. coli*-Produced Cry3A Protein	The method of preparing by fermentation the delta endotoxin from *B.t.t.* in *E. coli* was presented. The protein was characterized for purity and stability after purification. This data indicates that normal fermentation techniques were used to produce the plant equivalent, microbial Cry3A delta endotoxin. CLASSIFICATION: ACCEPTABLE	429322-04
Protein Characterization and Expression	The Cry3A delta endotoxin as expressed in potato tissue or an *E. coli* alternative gives a similar immunoreactivity and electrophoretic mobility to registered microbial products producing the same delta endotoxin. CLASSIFICATION: ACCEPTABLE	429322-06

B. HUMAN HEALTH ASSESSMENT

1. Background

The basis for the toxicology assessment is the fact that all the *Bt* plant-pesticides are proteins. Proteins are commonly found in the diet and, except for a few well described phenomena, present little risk as a mammalian hazard. In addition, for the majority of proteins currently registered, the source bacterium has been a registered microbial pesticide which has been approved for use on food crops without specific restrictions. Because of their use as microbial pesticides, a long history of safe use is associated with many *Bt* products.

Several types of data are required for the *Bt* plant-pesticides 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-pesticide 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-pesticides. 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. We expect to receive the final report and make any recommendations from the panel available to the public.

2. *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, but in the initial *Bt* products registered, gastric and intestinal fluids were examined separately. 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. All except one (Cry9C) were degraded in gastric fluid in 0-7 minutes. All the *Bt* proteins tested in intestinal fluid were not affected by trypsin digestion as would be expected since this is similar to their behavior in the insect gut. In intestinal fluid, those *Bt* plant-pesticides that are expressed as protoxin molecules broke down into the active toxin moiety and degraded no further.

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.

3. 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. For a few of the protein plant-pesticides registered to date there is information about the heat/processing stability of the delta-endotoxins as expressed in bioactivity or immunological recognition after typical food processing. Cry1Ab (Novartis) and Cry1Ac (Monsanto) were demonstrated to be inactive in processed corn or cottonseed meal. These results were used to justify waivers for the fish feed toxicity studies. Cry9C was shown to be stable after a 10 minute treatment at 90°C (MRID 442581-08 & 447143-05). No heat stability studies were available for Cry3A. Amino acid sequence homology comparisons for Cry1Ab, Cry1Ac and Cry3A against the database of known allergenic and toxic

proteins were not submitted. Cry 9C amino acid sequence homology comparisons did not yield any significant homologies for either toxin proteins or allergens (MRID 442581-09 & 443844-04).

4. Acute Oral Toxicity

The basis for addressing the toxicity of proteins 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-pesticides, even at relatively high dose levels in the acute oral exposure, the proteins are not considered toxic. The acute oral toxicity test is performed in mice with a pure preparation of the plant-pesticide protein at doses from 3280 to over 5000 mg/kg bodyweight. None of the tests performed to date have shown any significant effects on the treated animals.

5. EPA Recommendation

The mammalian toxicity data continue to support the registrations of the *Bt* products described. Although this does not affect the safety assessment for these products, some of the data that is currently required for registration of new products was not provided to support registration of some of the products under consideration here. In order to complete the database for these products, EPA will request that each company provide the indicated data/information on heat/processing stability and amino acid sequence homology comparisons to known protein toxins (full length comparison) and all allergens (stepwise 8 amino acid fragments comparison).

None of this data has a significant impact upon the safety of the product, but would bring each product up to current data requirement standards. None of the products registered to date, which have a tolerance for food use, show any characteristics of toxins or food allergens. However, if the June SAP report suggests that additional data are necessary, EPA will consider these recommendations prior to finalizing the current risk assessment.

Table B1 Studies Needed to Complete Product Database

Common Name and Cry Protein	OPP Chemical Code	Study Type
*Bt*11, Cry1Ab *Bt* Corn	006444	Amino Acid Sequence Homology
MON810, Cry1Ab *Bt* Corn	006430	Amino Acid Sequence Homology/ Processing and/or Heat Stability
Cry1Ac *Bt* Cotton	006445	Amino Acid Sequence Homology

6. Human Health Assessment of Cry1Ab Crops, Including But Not Limited To:

Bt11 Cry1Ab Bt Corn (006444) and MON810 Cry1Ab Bt Corn (006430)

a. Toxicology Assessment

Mammalian toxicology data are available to examine the potential effects of Cry1Ab on human health and assess if the data support the registration of *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production (plasmid vector pZO1502) in corn and *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production in corn (OPP PC code 006430). These data would also support other Cry1Ab plant-pesticides' human health assessments provided adequate information was submitted to show that the Cry1Ab proteins in question were biochemically and functionally similar to the proteins of the plant-pesticides already examined.

1) Acute Toxicity

Study Type	Result	MRID #
Acute Oral Toxicity Study of *B.t.k.* HD-1 Tryptic Core Protein In Albino Mice	No test substance related deaths occurred. One female died within a day of BSA dose administration due to a perforated trachea. The majority of the animals failed to gain weight or showed a slight weight reduction. No treatment related trends in these losses was apparent. CLASSIFICATION: ACCEPTABLE. Test substance is given a TOXICITY CATEGORY IV rating although highest dose administered is 4000 mg/kg due to lack of any evidence of a dose/effect relation.	434680-01

2) Mutagenicity and Developmental Toxicity, Subchronic Toxicity, and Chronic Exposure and Oncogenicity Assessment

Data demonstrating no mammalian toxicity at high levels of exposure confirm the safety of the product at levels well above any possible maximum exposure levels anticipated for a plant-pesticide. 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-pesticide was derived. [See 40 CFR Sec. 158.740(b).] For microbial products, further toxicity testing to verify the observed effects and clarify the source of the effects (Tiers II & III) and residue data are triggered by significant acute effects in studies such as the mouse oral toxicity study.

The acute oral toxicity data submitted support the determination that the Cry1Ab protein is 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). Since no effects were shown to be caused by the plant-pesticides, even at relatively high dose levels, the Cry1Ab delta-endotoxin protein is not

considered toxic. Therefore, the mutagenicity, developmental toxicity, subchronic toxicity, chronic exposure and oncogenicity assessment studies were not required.

3) Effects on the Immune System

Since Cry1Ab is a protein, allergenic potential was considered. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases, to be glycosylated and present at high concentrations in the food. Data has been submitted which demonstrates that the Cry1Ab delta-endotoxin is degraded in two minutes by gastric fluid *in vitro* and is non-glycosylated. Studies submitted to EPA done in laboratory animals have not indicated any potential for allergic reactions to *B. thuringiensis* or its components, including the delta-endotoxin in the crystal protein. Despite decades of widespread use of *Bacillus thuringiensis* as a pesticide (it has been registered since 1961), there have been no confirmed reports of immediate or delayed allergic reactions to the delta-endotoxin itself despite significant oral, dermal and inhalation exposure to the microbial product. Several reports under FIFRA § 6(a)2 have been made for various *Bacillus thuringiensis* microbial products claiming dermal allergic reactions. However, the Agency determined these reactions were not due to *Bacillus thuringiensis* itself or any of the Cry toxins. Thus, the Cry1Ab protein is not expected to be a food allergen.

Allergenicity Endpoints of Cry1Ab Crops [*Bt*11 and MON810 *Bt* Corn (006444 & 006430)]

Study Type	Result	MRID #
In vitro Digestibility	The tryptic core Cry1Ab protein is significantly degraded by 2 minutes incubation in gastric fluid but not significantly affected by 19.5 hours in intestinal fluid as monitored by western blot. The decease in bioactivity of these digestions against tobacco budworm is similar to its loss of immunorecogniton in western blots CLASSIFICATION: ACCEPTABLE	434392-01

4) Effects on the Endocrine System

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-pesticides at this time.

5) Dose Response Assessment

No toxicological endpoints were identified, therefore a dose response assessment was not required.

6) Dietary Risk Characterization

a) Toxicity and Allergenicity Conclusions

The data submitted and cited regarding potential health effects for the Cry1Ab protein include information on the characterization of the expressed Cry1Ab delta-endotoxin in corn, the acute oral toxicity, and *in vitro* digestibility of the delta-endotoxin.

Adequate information was submitted to show that the Cry1Ab test material derived from microbial cultures were biochemically and functionally similar to the proteins produced by the plant-pesticide 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 conclusion that the Cry1Ab protein is non-toxic to humans. Therefore, because no effects were shown to be caused by these plant-pesticides, even at relatively high dose levels (4000 mg/kg), the Cry1Ab delta-endotoxin protein is 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-pesticide was derived. [See 40 CFR Sec. 158.740(b).] Further toxicity testing to verify the observed effects and clarify the source of the effects (Tiers II & III) and residue data are only triggered by significant acute effects in studies such as the mouse oral toxicity study. Because the acute testing showed no toxicity, higher tier testing is not required.

Because Cry1Ab is a protein and the major exposure is dietary, food allergenic potential was considered. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases, are glycosylated and present at high concentrations in the food. Data has been submitted which demonstrates that the Cry1Ab delta-endotoxin is degraded in two minutes by gastric fluid *in vitro* and is non-glycosylated. After decades of widespread use of *Bacillus thuringiensis* as a pesticide (it has been registered since 1961), there have been no confirmed reports of immediate or delayed allergic reactions to the delta-endotoxin itself despite significant oral, dermal and inhalation exposure to the microbial product. Several reports under FIFRA § 6(a)2 have been made for various *Bacillus thuringiensis* microbial products claiming dermal allergic reactions. However, the Agency determined these reactions were not due to *Bacillus thuringiensis* itself or any of the Cry toxins. Thus, the Cry1Ab protein is not expected to be a food allergen.

Both (1) available information concerning the dietary consumption patterns of consumers (and major identifiable subgroups of consumers including infants and children) and (2) 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 because the lack of mammalian toxicity at high levels of exposure demonstrates the safety of the product at levels above possible maximum exposure levels.

The genetic material necessary for the production of the plant-pesticides active ingredients are the nucleic acids (DNA) which comprise (1) genetic material encoding these proteins and (2) their regulatory regions. "Regulatory regions" are the genetic material (termed promoters, terminators and enhancers) that control the expression of the DNA encoding proteins. DNA is 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.

Residue chemistry data were not required for a human health effects assessment of the subject plant-pesticide ingredients because of the lack of mammalian toxicity in the acute exposures.

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 provides that EPA shall apply an additional tenfold margin of exposure (safety) for infants and children in the case of threshold effects to account for pre- and post-natal toxicity and the completeness of the database unless EPA determines that a different margin of exposure (safety) will be safe for infants and children.

In this instance, based on all the available information, the Agency concludes that infants and children will consume only minimal residues of this plant-pesticide and that there is a finding of no toxicity.

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) Aggregate Exposure (Not Including Occupational Exposure) Risk Conclusions

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-pesticides chemical residue, and exposure from non-occupational sources. Exposure via the skin or inhalation is not likely since the plant-pesticides 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 drinking water. However, a lack of mammalian toxicity and the digestibility of the plant-pesticides has been demonstrated. The use sites for Cry1Ab delta endotoxin are all agricultural for control of lepidopteran 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.

d) Cumulative Effects Risk Conclusions

The Agency 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-pesticides, there are no cumulative effects.

e) Dietary Risk Conclusion

There is a reasonable certainty that no harm will result from aggregate exposure to the United States population, including infants and children, to the Cry1Ab protein and the genetic material necessary for its production. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. We have arrived at this conclusion because, as discussed above, no toxicity to mammals has been observed for the currently registered plant-pesticides.

f) Occupational Exposure and Risk Characterization

Exposure via the skin or inhalation is not likely since the plant-pesticides 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. If any unreasonable adverse effects caused by exposure to Cry1Ab are identified, these effects must be reported to the Agency as described in Sec. 6(a)(2) of FIFRA.

BPPD RECOMMENDATION:
There is a reasonable certainty that no harm will result from exposure to *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production in corn. This includes all anticipated dietary exposures and all other exposures for which there is reliable information.

7. Human Health Assessment of Cry1Ac Bt Crops, Including But Not Limited To Cry1Ac Bt Cotton (6455)

a. Toxicology Assessment

Mammalian toxicology data are available to examine the potential effects of Cry1Ac on human health and assess if the data support the support registration of *Bacillus thuringiensis* Cry1Ac delta-endotoxin and the genetic material necessary for its production in corn and *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production in cotton. These data would also support other Cry1Ac plant-pesticides' human health assessments provided adequate information was submitted to show that the Cry1Ac test material derived from microbial cultures were biochemically and, functionally similar to the proteins produced by the plant-pesticides ingredients.

1) Acute Toxicity

The acute oral toxicity data demonstrates that the Cry1Ac endotoxin is non-toxic to humans.

Toxicological Endpoints of Cry1Ac Crops

Study	**Result**	**MRID #**
Acute Oral Toxicity	Ten male and female CD-1 mice per dose level were exposed by oral gavage to 500, 1000 and 4200 mg/kg bodyweight of *E. coli* produced *B.t.k.* HD-73 toxin. The controls were given the protein equivalent of 6340 mg/kg of bovine serum albumin. No mortalities or treatment related adverse effects were seen in either the treated or control mice. There were no observable dose related effects seen upon necropsy. CLASSIFICATION: ACCEPTABLE. TOXICITY CATEGORY IV.	431452-13

2) Mutagenicity and Developmental Toxicity, Subchronic Toxicity, and Chronic Exposure and Oncogenicity Assessment

The lack of mammalian toxicity at high levels of exposure demonstrates the safety of the product at levels above possible maximum exposure levels. 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-pesticide was derived. [See 40 CFR Sec. 158.740(b).] For microbial products, further toxicity testing to verify the observed effects and clarify the source of the effects (Tiers II & III) and residue data are only triggered by significant acute effects in studies such as the mouse oral toxicity study.

The acute oral toxicity data submitted support the determination that the Cry1Ac protein is 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). Since no effects were shown to be caused by the plant-pesticides, even at relatively high dose levels, the Cry1Ac delta-endotoxin protein is not considered toxic. Therefore, the mutagencity, developmental toxicity, subchronic toxicity, chronic exposure and oncogenicity assessment studies are not required.

3) Effects on the Immune System

Since Cry1Ac is a protein, allergenic potential was considered. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases, are glycosylated and present at high concentrations in the food. Data has been submitted which demonstrates that the Cry1Ac delta-endotoxin is degraded between two minutes (MRID# 439995-03) and seven minutes by gastric fluid *in vitro* and is non-glycosylated. Studies submitted to EPA done in laboratory animals have not indicated any potential for allergic reactions to *B. thuringiensis* or its components, including the delta-endotoxin in the crystal protein. After decades of widespread use of *Bacillus thuringiensis* as a pesticide (it has been registered since 1961), there have been no confirmed reports of immediate or delayed allergic reactions to the delta-endotoxin itself despite significant oral, dermal and inhalation exposure to the microbial product. Several reports under FIFRA § 6(a)2 have been made for various *Bacillus thuringiensis* microbial products claiming dermal allergic reactions. However, the Agency determined these reactions were not due to *Bacillus thuringiensis* itself or any of the Cry toxins. Thus, the Cry1Ac protein is not expected to be a food allergen.

Allergenicity Endpoints of Cry1Ac Crops

Study	Result	MRID #
In vitro Digestibility	The *B.t.k.* HD-73 protein was rapidly degraded to fragments not recognized in a western blot after 7 minutes incubation in simulated gastric fluid (SGF) and was not active in a tobacco budworm (TBW) bioassay after SGF incubation. The *in vitro* digestibility assay provides useful information to predict the metabolic fate of the Cry1Ac protein and its potential as a food allergen. However, it is not clear how this protein assay's results relate to protein toxicity. Therefore the Agency also requested that an acute oral toxicity study be done to confirm the expected lack of toxicity indicated by the *in vitro* digestibility results. CLASSIFICATION: ACCEPTABLE	431452-14

4) Effects on the Endocrine System

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-pesticides at this time.

5) Dose Response Assessment

No toxicological endpoints are identified so no dose response assessment is required.

6) Dietary Risk Characterization

a) Toxicity and Allergenicity Conclusions

The data submitted and cited regarding potential health effects for the Cry1Ac protein include information on the characterization of the expressed Cry1Ac delta-endotoxin in cotton, the acute oral toxicity, and *in vitro* digestibility of the delta-endotoxin. The results of these studies were determined to be adequate to evaluate human risk and the validity, completeness, and reliability of the available data from the studies were considered.

Data was submitted to show that the Cry1Ac test material derived from microbial cultures were biochemically and, functionally similar to the proteins produced by the plant-pesticide ingredients. Production of microbially-produced protein was chosen in order to obtain sufficient material for testing.

The acute oral toxicity data submitted supports the determination that the Cry1Ac protein is non-toxic to humans. Therefore, since no effects were shown to be caused by the plant-pesticides, even at relatively high dose levels (5,000 mg/kg), the Cry1Ac delta-endotoxin protein is 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-pesticide was derived. [See 40 CFR Sec. 158.740(b).] Further toxicity testing to verify the observed effects and clarify the source of the effects (Tiers II & III) and residue data are only triggered by significant acute effects in studies such as the mouse oral toxicity study. Because the acute testing showed no toxicity, higher tier studies are not required.

Because Cry1Ac is a protein and the major exposure is dietary, food allergenic potential was considered. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases, are glycosylated and present at high concentrations in the food. Data has been submitted which demonstrates that the Cry1Ac delta-endotoxin is degraded between two minutes (MRID#439995-03) and seven minutes by gastric fluid *in vitro* and is non-glycosylated. Despite decades of widespread use of *Bacillus thuringiensis* as a pesticide (it has been registered since 1961), there have been no confirmed reports of immediate or delayed allergic reactions to the delta-endotoxin itself despite significant oral, dermal and inhalation exposure to the microbial product. Several reports under FIFRA § 6(a)2 have been made for various *Bacillus thuringiensis* products claiming allergic reactions. However, the Agency determined these reactions were not due to *Bacillus thuringiensis* itself or any of the Cry toxins. Thus, the Cry1Ac protein is not expected to be a food allergen.

Although Cry1Ac expression level data was required for an environmental fate and effects assessment, residue chemistry data were not required for a human health effects assessment of the subject plant-pesticide ingredients because of the lack of mammalian toxicity.

Both (1) available information concerning the dietary consumption patterns of consumers (and major identifiable subgroups of consumers including infants and children) and (2) 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 because the lack of mammalian toxicity at high levels of exposure demonstrate the safety of the product at levels above possible maximum exposure levels.

The genetic material necessary for the production of the plant-pesticides active ingredients are the nucleic acids (DNA) which comprise (1) genetic material encoding these proteins and (2) their regulatory regions. "Regulatory regions" are the genetic material (termed promoters, terminators and enhancers) that control the expression of the DNA encoding proteins. DNA is 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 provides that EPA shall apply an additional tenfold margin of exposure (safety) for infants and children in the case of threshold effects to account for pre- and post-natal toxicity and the completeness of the database unless EPA determines that a different margin of exposure (safety) will be safe for infants and children.

In this instance, based on all the available information, the Agency concludes that infants and children will consume only minimal residues of this plant-pesticide and that there is a finding of no toxicity.

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) Aggregate Exposure (Not Including Occupational Exposure) Risk Conclusions

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-pesticides chemical residue, and exposure from non-occupational sources. Exposure via the skin or inhalation is not likely since the plant-pesticides 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 products and drinking water. However, a lack of mammalian toxicity and the digestibility of the plant-pesticides has been demonstrated. The use sites for Cry1Ac delta endotoxin are all agricultural for control of lepidopteran 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.

d) Cumulative Effects Risk Conclusions

The Agency 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-pesticides, there are no cumulative effects.

e) Tolerance 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 Cry1Ac protein and the genetic material necessary for its 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-pesticides. As a result, EPA established an exemption from tolerance requirements pursuant to FFDCA section 408(j)(3) for *Bacillus thuringiensis* Cry1Ac delta-endotoxin and the genetic material necessary for its production in all plants.

Bacillus thuringiensis subspecies *kurstaki* Cry1Ac delta-endotoxin and the genetic material necessary for its production in all plants are exempt from the requirement of a tolerance when used as plant-pesticides in all plant raw agricultural commodities. ``Genetic material necessary for its production" means the genetic material which comprise (1) genetic material encoding the Cry1Ac delta-endotoxin and (2) its regulatory regions. ``Regulatory regions" are the genetic material that control the expression of the genetic material encoding the Cry1Ac delta-endotoxin, such as promoters, terminators, and enhancers.

f) Occupational Exposure and Risk Characterization

Exposure via the skin or inhalation is not likely since the plant-pesticides 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 Cry1Ac are identified, these effects must be reported to the Agency as described in Sec. 6(a)(2) of FIFRA.

BPPD RECOMMENDATION:

There is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the Cry1Ac protein and the genetic material necessary for its 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 no toxicity to mammals has been observed for the plant-pesticides and anticipated exposures are negligible.

8. Human Health Assessment of Cry9C Corn (006466)

a) Toxicology Assessment

1) Acute Toxicity

Toxicological Endpoints of Cry9C Corn

Study	Result	MRID #
An Acute Oral Toxicity in Mice with Cry9C Protein as Purified from *Bacillus thuringiensis* Cry9C.PGS2	There were no deaths in any test animals due to test material given at the dose of 3,760 mg/kg during the 14 day observation period. One male mouse displayed hair loss between days 2 and 5. One female displayed decreased activity on the day of dosing. Another female displayed decreased activity, wobbly gait, decreased feces and felt cool to the touch. A third female displayed decreased feces on day 1. All the male mice gained weight during the test period (except during the pre-dosing fast period). Two female mice failed to gain weight between day 0 (prefasted weight) and day 7 and three failed to gain weight between day 7 and day 14. One female did not recover her pre-fasting body weight by day 14. CLASSIFICATION: ACCEPTABLE	442581-07

2. Mutagenicity and Developmental Toxicity, Subchronic Toxicity, and Chronic Exposure and Oncogenicity Assessment

The lack of mammalian toxicity at high levels of exposure demonstrates the safety of the product at levels above possible maximum exposure levels. 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-pesticide was derived. [See 40 CFR Sec. 158.740(b).] For microbial products, further toxicity testing to verify the observed effects and clarify the source of

the effects (Tiers II & III) and residue data are only triggered by significant acute effects in studies such as the mouse oral toxicity study.

The acute oral toxicity data submitted support the determination that the Cry9C protein 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). Since no effects were shown to be caused by the plant-pesticides, even at relatively high dose levels, the Cry9C protein is not considered toxic. Therefore, the mutagencity, developmental toxicity, subchronic toxicity, chronic exposure and oncogenicity assessment studies are not required.

3) Allergenicity

Since Cry9C is a protein, allergenic potential was considered. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases, may be glycosylated (have a carbohydrate group attached) and present at high concentrations in the food. Data has been submitted which demonstrates that the Cry9C protein is present in low concentrations in corn. However, it is resistant to degradation by heat, acid, and proteases. Additionally, although staining for the carbohydrates did not reveal any detectable Cry9C protein glycosylation in the data presented, the company claims there is some minor positive staining for the presence of carbohydrates. The best available information on the uptake of intact proteins from the diet would indicate that the intact Cry9C protein would not be available in products from animals fed corn products containing Cry9C protein. In addition, homology searches indicated a lack of homology to known toxins or allergens.

Allergenicity Endpoints of Cry9C Corn

Study Type	Result	MRID #
In vitro Digestibility and Heat Stability of the Endotoxin Cry9C of *Bacillus thuringiensis*	The samples of lyophilized Cry9C protein expressed in corn showed no signs of protein disintegration when subjected to *in vitro* digestion in simulated mammalian gastric fluid. These digestions were done either with of without pepsin in the low pH buffer (pH 2.0) and were assayed by western blot from samples taken at several time points from the mixing the reagents to after 4 hours exposure to the digestive fluids. The same 70kD double band seen in the original Cry9C protein in plant tissue at time 0 was also seen, undiminished , in all the subsequent incubation samples. No effect on Cry9C activity as determined by bioassay was seen after any heat treatment. The most stringent heat treatment was 90°C for 10 minutes. CLASSIFICATION: ACCEPTABLE.	442581-08

Cry9C *Bacillus thuringiensis* Insecticidal Protein Identification of Sequence Homology with Allergenicity by Searching Protein Databanks	Sequence identity for any of the eight amino acid regions in Cry9C was found only to other *Bt* crystal proteins. No match between any 8 amino acid sequence in Cry9C and any of the allergenic proteins known in the SWISS protein database was found. This lack of homology at a finer level of examination is further evidence that Cry9C is not related to known allergens using a structural consensus approach. CLASSIFICATION: ACCEPTABLE.	443844-04
Study Type	**Result**	**MRID #**
Investigation of Allergens in Wild-Type and Transgenic Corn	The 21 sera samples from suspected corn-sensitive individuals all tested positive in the RAST assay by having >3% reactivity. The transgenic and wild-type aqueous corn extracts were not obviously different in responsiveness for individuals and a t-test of the RAST % reactivity did not reveal any significant differences. The RAST inhibition assay gave results indicating that both wild type and transgenic corn extracts gave substantial inhibition of the wild type corn RAST. Statistical analysis of the inhibition curves generated for RAST inhibition from wild type versus transgenic corn extracts did not indicate significantly different 50% inhibition values, slopes or y-intercepts. The type of extract, aqueous or alcoholic, utilized in the inhibition assays was never specified. Both the wild type and transgenic aqueous corn extracts gave higher levels of reactivity in the immunoblot assay than the alcoholic extracts. A comparison of the IgE reactions for specific corn atopic individuals indicated that there were similar reactive banding patterns in both transgenic and wild type corn. In some individuals there were a greater number of reactive bands ranging in molecular weight whereas in others there were only one or two bands, generally of lower molecular weight, which had very significant staining. There was no identification of individuals in the SDS-PAGE lanes so no correlation between the intensity of the % reactivity in RAST and the number or intensity of staining in the immunoblot assay could be made. A two-fold dilution series with a pool of 10 RAST positive corn atopic sera was tested against the wild type and transgenic corn extracts. The pattern of reactivity was very similar between the transgenic and wild type extracts with the intensity of the reaction again being higher for the aqueous versus alcoholic extracts. There were some unique bands present in either the wild type or transgenic extracts but since these bands did not show detectable effects on the serum reactivity kinetics in the RAST or RAST inhibition assays it is difficult to judge the importance of their presence. CLASSIFICATION: SUPPLEMENTAL. This study does not address the potential for inducing food allergy from a novel protein lacking a history of dietary exposure. An additional control testing purified Cry9C protein against corn atopic sera should have been included to establish the negative reactivity background. The study does establish a baseline of corn allergen reactivity for subsequent comparisons if such an allergic response does occur over time.	443844-05

Study Type	Result	MRID #
Amino Acid Sequence Homology Search with the Corn Expressed Truncated Cry9C Protein Sequence	Three hundred sequences were listed as having regions of homology with the 626 amino acids of the Cry9C truncated toxin protein. The first 64 proteins in the list were all parasporal proteins from *Bacillus thuringiensis* otherwise known as delta-endotoxins. Other delta-endotoxins were found at 67, 76, 78, 79, and 80. These proteins had regions of homology that gave a "significant homology". The table of values indicated a matching score above 4 standard deviations would contain all the delta-endotoxins mentioned above. The algorithm for converting the matches and penalties into homology scores was not described although it was stated that " all other proteins (besides the delta-endotoxins referred to above) have less than 20% exact sequence matching and no major stretches of sequence homology could be detected, indicating that in these cases the sequence homology is not significant." CLASSIFICATION: ACCEPTABLE.	442581-09

4) Effects on the Endocrine System

The pesticidal active ingredient is a protein, 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 this plant-pesticide at this time.

5) Dose Response Assessment

No toxicological endpoints are identified, therefore a dose response assessment was not required.

6) Dietary Exposure and Risk Characterization

Based on the toxicology data cited and the limited exposure expected with animal feed use, there is reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to residues of *Bacillus thuringiensis* subspecies *tolworthi* Cry9C protein and the genetic material necessary for its production in corn used as animal feed. This includes all anticipated dietary exposures to animal products and all other exposures for which there is reliable information. The Agency has arrived at this conclusion because the tolerance exemption is limited to feed use only. The conclusion of safety is supported by the lack of toxicity after administration of a high oral dose (3,760 mg/kg), the lack of homology to known toxins or allergens, and the minimal to nonexistent exposure via dietary and non-dietary routes.

a) Aggregate Exposure

The available information on the aggregate exposure levels of consumers (and major identifiable subgroups of consumers) to the Cry9C protein residue include dietary exposure and exposure from non-occupational sources. Exposure via the skin or inhalation is not likely since the Cry9C plant-pesticide is contained within plant cells essentially eliminating these exposure routes or reducing these exposure routes to negligible. Drinking water is unlikely to be significantly contaminated with Cry9C protein due to the low expression of the protein in corn tissue, degradation of plant materials in the soil and low leaching potential of a protein from a soil matrix. Minimal to non-existent oral exposure could occur from drinking water or from ingestion of meat, poultry, eggs or milk from animals fed corn containing the plant-pesticide. While unlikely, meat, eggs or milk from animals fed corn containing the plant-pesticide could contain negligible but finite residues. This is viewed as a remote possibility due to the low Cry9C expression level in corn tissue (3 to 250 microgram/gm dry weight), the anticipated degradation and elimination of the Cry9C protein by the animal or the lack of uptake of such a large protein by the animal's intestinal tract. It is not possible to establish with certainty whether finite residues will be incurred, but there is no reasonable expectation of finite residues. However, the best available information on the uptake of intact proteins from the diet would indicate that the intact Cry9C protein would not be available in products from animals fed corn products containing Cry9C protein.

b) Cumulative Effects

The Agency 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 adults as well as on infants and children of such residues and other substances with a common mechanism of toxicity. Since there is no indication of mammalian toxicity to the Cry9C protein from the studies submitted, there is no reason to believe there would be cumulative toxic effects.

c) Safety Determination

The tolerance exemption is limited to residues of the Cry9C protein resulting from feed use only. The basis of safety for this tolerance exemption includes both the results of the acute oral study at high doses indicating no toxicity and the anticipated minimal to nonexistent human dietary exposure of the Cry9C protein via animal feed use. *Bt* microbial pesticides, containing Cry proteins other than Cry9C, have been applied for more than 30 years to food and feed crops consumed by the U.S. population. There have been no human safety problems attributed to the specific Cry proteins. An oral dose of the tryptic core Cry9C protein of at least 3,760 mg/kg was administered to 10 animals without mortality, thus demonstrating a lack of toxicity for the protein. Transient weight loss in three female rodents was observed, but not in any males. Transient weight loss has been observed in similar studies conducted on other purified Cry proteins as well as microbial pesticides and this is not considered a toxic endpoint for risk assessment purposes.

A comparison of the amino acid sequence of the Cry9C protein with those found in the PIR, Swiss-Prot and HIV AA data bases did not reveal any significant homology with known toxins or allergens. The *in vitro* digestibility study showed the Cry9C protein to be stable to pepsin at pH 2.0. The Cry9C protein was shown to be stable to heat at 90 degrees C for 10 minutes. The Cry9C protein in corn is similar to the trypsin resistant core and should therefore be stable to tryptic digest; however, no data was submitted to demonstrate this. The best available information to date would indicate that edible products derived from animals such as meat, milk and eggs, intended for human consumption, have not been shown to be altered in their allergenicity due to changes in the feed stock utilized. This information would include no transfer of allergenic factors from cattle fed soybeans or corn to the derived meat or milk eaten by individuals with food sensitivity to soybeans or corn.

d) Infants and Children

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 provides that EPA shall apply an additional tenfold margin of exposure (safety) for infants and children in the case of threshold effects to account for pre- and post-natal toxicity and the completeness of the database unless EPA determines that a different margin of exposure (safety) will be safe for infants and children. In this instance, based on all the available information, the Agency concludes that infants and children will consume only minimal residues of this plant-pesticide and that there is a finding of no toxicity. 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.

7) Occupational Exposure and Risk Characterization

Exposure via the skin or inhalation is not likely since the plant-pesticides 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 Cry9C are identified, these effects must be reported to the Agency as described in Sec. 6(a)(2) of FIFRA.

BPPD RECOMMENDATION:
There is a reasonable certainty that no harm will result from exposure to *Bacillus thuringiensis* subspecies *tolworthi* and the genetic material necessary for its production in corn used for animal feed. Cry9C is approved for used in animal feed only and strict guidelines have been imposed to prevent Cry9C containing corn from entering the human food supply. The EPA has determined that the Cry9C protein would not likely cause an allergic reaction to man when used in feed corn because: (1) it was not from allergenic sources and (2) the best available information indicates that edible products derived from animals such as meat, milk and eggs, intended for human consumption, have not been shown to be altered in their allergenicity due to changes in the feed stock utilized. For example, despite the fact that soybeans contain important clinical allergenic proteins that are stable to gastric fluids and high temperatures, the allergenic properties are not transferred into the meat, milk or eggs of animals fed soybean products and thus, show no adverse effects when eaten by soybean-sensitive individuals. The Agency is reviewing a petition from Adventis for a tolerance exemption for Cry9C in food commodities.

9. Human Health Assessment of Cry3A Potatoes

a. Toxicology Assessment

The delta endotoxin proteins of *B. thuringiensis* have been intensively studied and no indications of mammalian toxicity have been reported. *Bt* microbial pesticides, containing Cry proteins other than Cry3A, have been applied for more than 30 years to food and feed crops consumed by the U.S. population. Furthermore, *B. thuringiensis* products containing Cry3A have been registered and in use for more than a decade, and the Agency has not received any reports of dietary toxicity attributable to their use. Therefore, the Agency does not anticipate any mammalian toxicity from this protein in plants based on the use history of *B. thuringiensis* products.

The data submitted by Monsanto indicate that this protein would be non-toxic to mammals under the proposed use. Cry3A protein was non-toxic to mice at doses up to 5220 mg/kg bodyweight. This level is >10,000 times the amount found in potato tubers. Adequate information was submitted to show that the test material derived from microbial cultures was essentially identical to the protein as produced by the potatoes. Production of a microbial Cry 3A delta endotoxin equivalent to plant-produced delta endotoxin was chosen in order to obtain sufficient material for mammalian testing. In addition, the *in vitro* digestibility studies indicate the protein was degraded within 30 seconds in simulated gastric fluid.

The genetic material necessary for the production of the *Bt* Cry3A delta endotoxin are the nucleic acids (DNA and RNA) which comprise the Cry3A gene and its controlling sequences. DNA and RNA are common to all forms of life, including plants, and the Agency knows of no instance where these nucleic acids have been associated with toxic effects related to the consumption 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 *Bt* Cry3A delta endotoxin in potatoes.

Toxicological Endpoints of Cry3A Crops

Study	Result	MRID #
Acute Oral Toxicity of *B.t.t.* Protein	*Bt* Cry3A delta endotoxin was not toxic by oral gavage when mice were dosed with up to 5220 mg/kg body weight. CLASSIFICATION: ACCEPTABLE These results placed this protein in TOXICITY CATEGORY IV.	429322-17

b. Allergenicity

After decades of widespread use of *Bacillus thuringiensis* as a pesticide (it has been registered since 1961), there have been no confirmed reports of immediate or delayed allergic reactions from exposure. Such incidents, should they occur, are required to be reported under FIFRA section 6(a)(2) and as a data requirement for registration of microbial pesticides (40CFR 158.740 and Subdivision M of the FIFRA testing guidelines, NTIS # PB89-211676).

Recently submitted *in vitro* studies confirm that the delta endotoxin would be readily digestible *in vivo*. Other studies on related proteins are consistent.

Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases, are glycosylated and present at high concentrations in the food. The company has submitted data to indicate that the *Bt* Cry3A delta endotoxin is degraded within 30 seconds by gastric fluid *in vitro*, is not present as a major component of food, and is apparently non-glycosylated when produced in plants.

Based on this information discussed above, the Agency concludes that Cry3A shows no characteristics of an allergen.

Allergenicity Endpoints of Cry3A Crops

Study Type	Result	MRID #
In-Vitro Digestibility	The 68 kD and 55kD *Bt* Cry3A proteins degraded within 30 seconds in simulated gastric fluid when analyzed by western blot and were not active against Colorado potato beetle after degradation. The 68kD *Bt* Cry3A protein degraded to 55kD within 2 hours of incubation in simulated intestinal fluid. The 55 kD form remained unchanged after 14 hours of incubation and retained its bioactivity and western blot results. These results indicate that, following ingestion by humans, the *Bt* Cry 3A proteins very likely will be degraded like other proteins to amino acids and peptides like other dietary proteins. CLASSIFICATION: ACCEPTABLE	429332-18

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