UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MAR 13 2009

MEMORANDUM

SUBJECT: BPPD Review of SmartStax Plant-Incorporated Protectant (PIP) (Decision No: 394799; DP Barcode: 355689; Submission 830991; MRID No's.: 474449-01 thru 07).

FROM: John L. Kough, Ph.D.
Microbial Pesticides Branch, Biopesticides and Pollution Prevention Division (7511P)

TO: Mike Mendelsohn, Regulatory Action Leader
Microbial Pesticides Branch, Biopesticides and Pollution Prevention Division (7511P)

ACTION REQUESTED: Review the data on DNA and protein expression submitted to confirm stability and similar protein levels of multiple PIP product to single event parentals.

BACKGROUND: Dow AgroScience has registered a corn product expressing Cry1F (EPA reg. no. 68467-2), Cry34ab1 and Cry35Ab1 (EPA reg. no. 68467-5) for control of both lepidopteran and coleopteran pests. Monsanto has registered a corn product expressing Cry2Ab2, Cry1A.105 (EPA reg. no. 524-575) and Cry3Bb1 (EPA reg.no. 524-551) for control of both lepidopteran and coleopteran pests. These two combination PIP products have been traditionally bred together to provide higher levels of pest control and decrease the likelihood of developing pest insect resistance.

CONCLUSION: The data on the DNA stability from Southern blot analysis and protein expression indicate that the DNA of the traits is stable and the hybrids express the different PIP proteins at similar levels compared to the single event parental lines.

RECOMMENDATION: Clarification of a minor point in the submission, not affecting the DNA stability and similar protein expression level determination, should be provided. This includes:

1) Consideration of safety of potential combined residues of glufosinate ad glyphosate herbicides used on this PIP product.
2) Discussion of the discrepancy between the size of the *Sac*I fragment indicated by plasmid map of PHP17661 and the *cry34Ab1* positive band in the southern blot.

DATA REVIEW RECORD

Active Ingredient: Cry1a.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry 34ab1, Cry35Ab1
Product Name: SmartStax corn
Company Name: Monsanto Company and Dow AgroSciences
Decision No.: 394799
DP Barcode: 355689
Submission No: 830991
MRID No: 474449-01 thru 07

SUMMARY OF DATA SUBMITTED:

474449-01: The molecular identities for MON 89034, TC1507, MON 99017, and DAS-59122-7 were confirmed and stable in the combined trait product. The expression levels of the introduced proteins were consistent with the levels in the single event plants. Laboratory assays using sensitive target species found no antagonistic or synergistic interaction among the Cry proteins in MON 89034 x TC1507 x MON 88017 x DAS-59122-7. As a result, environmental and health assessments conducted for the single event products can be applied to the combined trait product. Field trials have established that the insecticidal efficacy of the combined trait product against lepidopteran and corn rootworm pests is consistent with combining the independent activities of each of the single event products in an additive manner. Non-target organism studies conducted for each single event, together with a confirmatory assay for the combined trait product, confirm that there is no unexpected interaction among the Cry proteins produced by the combined trait product. Prior assessments of each individual event and bridging analyses for the combined trait product indicate that MON 89034 x TC1507 x MON 88017 x DAS-59122-7 will not have adverse effects on human health or the environment. The safety of the combined residues of glufosinate and glyphosate should be addressed.
CLASSIFICATION: Supplemental. Summary information only.

474449-02: The results of Southern blot analyses showed that the hybridization patterns of Southern blot analysis for the combined train corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 were identical to the respective single products MON 89034, TC1507, MON 88017, and DAS-59122-7.
CLASSIFICATION: Supplemental. Summary information only.

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474449-03: Southern blot analysis confirmed in the combined trait corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 the presence of sequences identical to sequences derived from MON 89034 and MON 88017.
CLASSIFICATION: Acceptable.

474449-04: Southern blot analysis confirmed the presence of TC1507 and DAS-59122-7 in the combined trait corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7. Hybridization patterns for the combined trait product were identical to those of the parental lines with *cry*1F, *cry*34Ab1, *cry*35Ab1, and the *pat* gene probes indicating that the TC1507 and DAS-59122-7 insertions were unaffected by combining with MON 89034 and MON 88017 through conventional breeding.
CLASSIFICATION: Acceptable.

474449-05: Levels of the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, CP4 EPSPS, Cry34Ab1, Cry35Ab1, and PAT proteins were determined by validated enzyme linked immunosorbent assay in corn tissues collected from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 produced at five US field sites in 2006. In the same manner, levels of the Cry1Ab.105 and Cry2Ab2 proteins in MON 89034, the Cry1F and PAT proteins in TC1507, the Cry3Bb1 and CP4 EPSPS proteins in MON 88017, and the Cry34Ab1, Cry35Ab1, and PAT proteins in DAS-59122-7 were determined in tissues of these corns grown alongside MON 89034 x TC1507 x MON 88017 x DAS-59122-7. The mean levels of each protein found in tissues from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 and the respective single trait corns support the conclusion that the levels of Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, and Cry35Ab1 proteins produced in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 are comparable to the corresponding levels produced by the appropriate single product corns. The levels of PAT in the combined trait product were as expected for the combined expression in TC1507 and DAS-59122-7, and the level of CP4 EPSPS produced in the combined trait product were similar to the level produced in MON 88017.
CLASSIFICATION: Supplemental. Summary information only.

474449-06: MON 89034 x TC1507 x MON 88017 x DAS-59122-7 is a combined trait corn that produces lepidopteran-active and coleopteran-active *Bacillus thuringiensis* (*Bt*) proteins, as well as the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) to confer tolerance to glyphosate herbicides. The levels of the lepidopteran-active Cry1A.105, Cry2Ab2, Cry3Bb1 proteins and the CP4 EPSPS protein were determined in tissues from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 plants grown at five US field sites in 2006. The test also included a conventional corn as a negative control and MON 89034 and MON 88017 corns as positive controls. Leaf, root, and whole plant samples were collected over the growing season, as well as pollen and grain samples at the appropriate times. The samples were extracted and analyzed using enzyme-linked immunosorbent assays. The levels of the Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS proteins in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn were comparable to those in the appropriate MON 88017 or MON 89034 positive control.
CLASSIFICATION: Acceptable.

474449-07: The levels of the coleopteran-active *Bacillus thuringiensis* (*Bt*) proteins Cry34Ab1, Cry35Ab1, and Cry1F, and the PAT protein were determined in tissues from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 plants grown at five US field sites in 2006. The test also included a conventional corn as a negative control and TC1507 and DAS-5912207 parental event corn as positive controls. Leaf, root, and whole plant samples were collected over the growing season, as well as pollen and grain samples at the appropriate times. The samples were extracted and analyzed using enzyme-linked immunosorbent assays (ELISA). The results indicate that the levels of Cry34Ab1, Cry35Ab1, and Cry1F in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 were comparable to the levels produced in the appropriate TC1507 or DAS-59122-7 control corn. The level of PAT in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 was higher in the combined trait products compared to TC1507 and DAS-59122-7, likely due to the presence of multiple copies of the *pat* gene in the stacks (one from each of the DAS parent lines). CLASSIFICATION: Acceptable.

DATA EVALUATION RECORD

Primary reviewer: Eric B. Lewis, M.S., Contract Reviewer, Oak Ridge National Labs
EPA Secondary Reviewer: John L. Kough, Ph.D., Biologist

STUDY TYPE:	Human Health and Environmental Assessment (Nonguideline)
MRID NO:	47444901
DP BARCODE:	DP355690
DECISION NO:	394799
SUBMISSION NO:	830991
TEST MATERIAL:	MON 89034 x TC1507 x MON 88017 x DAS-59122-7 (a.i., Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1)
STUDY NO:	MSL0021223, File no. 07-CR-192E-1
SPONSOR:	Monsanto Company, 800 North Lindbergh Blvd., St. Louis, MO 63167
TESTING FACILITY:	N/A
TITLE OF REPORT:	Human Health and Environmental Assessment of the Plant-Incorporated Protectant *Bacillus thuringiensis* Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, and Cry35Ab1 Proteins Produced in the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7
AUTHOR:	Burns, J.A.
STUDY COMPLETED:	June 11, 2008
CONFIDENTIALITY CLAIMS:	None.
GOOD LABORATORY PRACTICE:	A signed and dated compliance statement was provided. The report comprises an assessment of data and information from other sources and was therefore not developed in compliance with 40 CFR Part 160.
STUDY SUMMARY:	MON 89034 x TC1507 x MON 88017 x DAS-59122-7 is a combined trait corn product produced by conventional breeding of the registered products MON 89034 x MON 88017 and TC1507 x DAS-59122-7. MON 89034 x TC1507 x MON 88017 x DAS-59122-7 expresses the Bt Cry proteins Cry1A.105, Cry2Ab2, and Cry1F, which provide protection against lepidopteran pests, and Cry3Bb1, Cry34Ab1, and Cry35Ab1, which provide protection against corn rootworm pests. The combined trait corn also contains the CP4 EPSPS and PAT proteins to provide tolerance to certain herbicides. The independent molecular identities for MON 89034, TC1507, MON 99017, and DAS-59122-7 were confirmed in the combined trait product, and the expression levels of the introduced proteins were consistent with the levels based on the single

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	event plants. Laboratory assays using sensitive target species found no antagonistic or synergistic interaction among the Cry proteins in MON 89034 x TC1507 x MON 88017 x DAS-59122-7. As a result, environmental and health assessments conducted for the single event products can be applied to the combined trait product. Field trials have established that the insecticidal efficacy of the combined trait product against lepidopteran and corn rootworm pests is consistent with combining the independent activities of each of the single event products in an additive manner. Non-target organism studies conducted for each single event, together with a confirmatory assay for the combined trait product, confirm that there is no unexpected interaction among the Cry proteins produced by the combined trait product. Prior assessments of each individual event and bridging analyses for the combined trait product indicate that MON 89034 x TC1507 x MON 88017 x DAS-59122-7 will not have adverse effects on human health or the environment. The safety of the combined residues of glufosinate and glyphosate should be addressed.
CLASSIFICATION:	**Supplemental. Summary Information only.**

Introduction

The combined trait corn MON 89034 x TC1507 x MON 88017 x DAS-59122-7 provides protection against lepidopteran and coleopteran insect pests. MON 89034 produces the lepidopteran–active *Bacillus thuringiensis* (*Bt*) Cry1A.105 and Cry2Ab2 proteins, and TC1507 produces the lepidopteran active *Bt* Cry1F protein. MON 88017 and DAS-59122-7 produce the coleopteran-active *Bt* Cry3Bb1 protein and the binary Cry34Ab1/Cry35Ab1 proteins, respectively.

Breeding and Manufacturing

Food and feed safety concerns are addressed by existing permanent exemptions from the requirement of a tolerance for each introduced protein in MON 89034 x TC1507 x MON 88017 x DAS-59122-7. Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, and Cry34Ab1/Cry35Ab1 are exempt in corn per 40 CFR 174.502, 174.520, 174.503, 174.519, and 174.506, respectively. Furthermore, MON 89034 x TC1507 x MON 88017 x DAS-59122-7 will be produced through conventional breeding the registered products MON 89034 x MON 88017 and TC1507 x DAS-59122-7. Protein interaction studies have confirmed the independent activity of the Cry proteins in the combined trait product. Therefore, no food and feed safety assessments have changed from the individual event assessments as a result of combining the events by conventional breeding. The food and feed assessments and tolerance exemptions for each event allow food and feed use of the combined trait product without restriction (FDA, 2001, 2004, 2005, 2007; EPA, 2001, 2005, 2008).

Environmental exposure of each single event was addressed during the reviews and product registrations for MON 89034, TC1507, MON 88017, and DAS-59122-7. Data provided to support registration of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 demonstrate the equivalence of Cry protein levels to the single events and demonstrate the lack of synergistic or antagonistic interactions between independent events and the Cry proteins, and no increased risk to endangered species (EPA, 2004b; Stillwell and Silvanovich, 2007; Phillips, 2008; Huesing and Levine, 2008; Levine et al., 2008; MacRae, 2008).

TC1507 x DAS-59122-7 and MON 89034 x MON 88017 are registered products previously assessed by EPA, and were not found to present any unexpected interactions (EPA, 2005j, 2008c). Therefore, they do not pose additional environmental or health risk. EPA determined that the mode of action for the *Bt* proteins in these combined trait products does not suggest an enhanced activity when they are combined. Data from similar studies confirm that there are no unexpected protein or event interactions in the traditionally bred combination of theses multiple PIP varieties MON 89034 x TC1507 x MON 88017 x DAS-59122-7. Therefore, the safety of the combined trait product is supported by analysis of each single event, and confirmed by the analysis of the final combined trait product.

Insect Resistance Management and Refuge

The introduction of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 with a 5% structured refuge in the US corn belt and a 20% structured refuge in cotton growing regions should produce sustained benefits for farmers and the environment. The product should provide improved insect resistance management (IRM) by providing a redundant and independent, season-long effective pesticidal activity against both the corn rootworm complex (CRW, *Diabrotica* spp.) and key lepidopteran corn pests.

Laboratory and field data support the combined use of Cry3Bb1 and Cry34/35Ab1 proteins for refuge reduction for CRW, and the use of Cry1A.105 and Cry2Ab2 plus Cry1F for lepidopteran control. Mathematical modeling provides evidence that the multiple effective dose combined trait product at 5% refuge will be at least as durable as, and likely more durable than, the individual single dose products (MON 88017 producing Cry3Bb1 or DAS-59122-7 producing Cry34/34Ab1) with a 20% refuge (Storer, 2008; Gustafson and Head, 2008).

Multiple Effective Dose Strategy

The strategy to combine two or more *Bt* proteins at effective doses with differing modes of action to markedly slow resistance development is the most effective approach for insect-protected biotechnology-derived crops (Roush, 1994, 1998). Use of multiple effective modes of action is also an established strategy to prolong effectiveness of agricultural herbicides and fungicides and medical tools (Gressel and Segel, 1990; Simone and Dooley, 1004; Wrubel and Gressel, 1995; NATAP, 1998; WHO, 2001; WHO/TDR, 2000; Bergstrom, et al., 2004; Beckie, 2006; Brent and Holloman, 2007).

The multiple effective dose strategy has been recognized by EPA as an approach for plant incorporated protectants (PIPs) that can reduce refuge requirements while extending expected longevity compared to single *Bt* products. This approach was used to reduce refuge size for dual effective dose *Bt* cotton products (MON 15985 and 281-24-236/3006-210-23) and for dual

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effective dose *Bt* corn products in cotton growing regions. The recent registration of MON 89034 confirms the independently active Cry1A.105 and Cry2Ab2 proteins as a dual effective dose product with complementary activity against lepidopteran pests in corn.

Data and information on sequence and structural dissimilarity between the rootworm-active proteins, differences in protein binding to CRW midgut epithelia, and insecticidal activity of Cry3Bb1 and Cry34/35Ab1 *in vivo* and *in vitro* support the lack of cross-resistance in the target pests for Cry3Bb1 and Cry34/35Ab1 (Li and Zhou, 2008; Zhuang, 2008; Head and Storer, 2008). Additionally, the mode of action of the Cry1F protein is different from the Cry1A.105 and Cry2Ab2 proteins based on structural and sequence differences, and studies of cross-resistance to Cry1F-resistant European corn borer (ECB, *Ostrinia nubilalis*) and fall armyworm (FAW, *Spodoptera frugiperda*) strains indicate incomplete to minimal cross-resistance to Cry1A.105, respectively, and no cross resistance to Cry1Ab2 (Schlenz et al., 2008). These studies indicate that the Cry proteins produced in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 represent different modes of action in controlling CRW and lepidopteran pests and are highly unlikely to be cross-resistant.

With multiple effective doses against both classes of pests, MON 89034 x TC1507 x MON 88017 x DAS-59122-7 is expected to be sustainable with a significantly smaller structured refuge than is needed for *Bt* field corn products containing only a single insecticidal protein against each pest. Current single gene *Bt* products have a 20% structured refuge in the US corn belt and a 50% refuge in cotton growing regions. Based on mathematical models (Gustafson and Head, 2008; Storer, 2008; Head and Storer, 2008), a 5% structured refuge in the corn belt and a 20% structured refuge in cotton growing regions where CEW is an important pest are expected to preserve the durability of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 for considerably longer than the existing refuges with single effective dose *Bt* corn products.

Insect Resistance Management Program

MON 89034 x TC1507 x MON 88017 x DAS-59122-7 represents two independent, effective modes of action against CRW and three modes of action against corn borers. It is expected to be significantly more durable than the existing single gene PIP products for CRW or lepidopteran control in corn. The primary IRM emphasis for MON 89034 x TC1507 x MON 88017 x DAS-59122-7 is management of the risk of resistance evolving to the western corn rootworm (WCRW, *Diabrotica virgifera*), although resistance management of ECB is also considered. From an IRM perspective, other target pests are a secondary consideration.

In the southern cotton growing states and counties, a 20% non-*Bt* corn refuge for MON 89034 x TC1507 x MON 88017 x DAS-59122-7 should be sufficient to manage the risk of resistance evolution in CEW. In regions where CEW and *Bt* cotton are not both present, a 5% refuge should be sufficient. In regions where WCRW, Northern corn rootworm (NCRW, *Diabrotica barberi*), and/or Mexican corn rootworm (MCRW, *Diabrotica virgifera zeae*) are important pests, the non-*Bt* corn refuge should be provided in the same field (strips at least four rows wide or as blocks) or in an adjacent field. In regions without corn rootworm pressure, the refuge should be planted within one-half mile of the MON 89034 x TC1507 x MON 88017 x DAS-59122-7 field. These distance requirements are the same as those for commercialized single gene corn products. Other key elements of the proposed IRM plan include implementation of a program similar to that for single gene *Bt* corn products.

Product Characterization

Data have been provided for molecular confirmation of identity, levels of the introduced proteins in selected tissues, equivalence of the combined trait product efficacy compared to single events, and the potential interaction of the pesticidal proteins in the combined trait product (Table 1). These data are consistent with the additive individual characteristics for MON 89034, TC1507, MON 88017, and DAS-59122-7, and confirm that the combined trait product MON 89034 x TC1507 x MON 88017 x DAS-59122-7 is as safe as the registered individual events.

Molecular Confirmation of Identity

The identity of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 was confirmed by southern blot analyses (Taylor et al., 2007; Schafer et al., 2008). There is no reason to suspect that conventional breeding of MON 89034, TC1507, MON 88017, and DAS-59122-7 would cause a molecular change. Each single event in the combined trait product was previously characterized via Southern blot analysis and reviewed by EPA (EPA 2005c, 2005e, 2005g, 2007, 2008b). The specific Southern blot hybridization patterns of the single event corn products comprising MON 89034 x TC1507 x MON 88017 x DAS-59122-7 were previously determined by Rice et al. (2006), Hondred (1998, 1999), Beasley et al. (2002), Locke (2003), and Weber (2004), respectively.

Protein Levels

The levels of the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, CP4 EPSPS, Cry34Ab1, Cry35Ab1 and PAT proteins in tissues of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 were assessed using validated enzyme linked immunosorbent assay (ELISA) (Stillwell and Silvanovich, 2007; Phillips, 2008). Four field locations across the US corn belt were used to generate the assayed tissues. The results are summarized in Table 2, and support the conclusion that the levels of the proteins produced in the combined trait product are similar to the levels of the corresponding proteins produced by each individual event. Minor differences observed were within the range of biological variability. The levels of PAT in the combined trait product were similar to the sum of the levels of PAT in TC1507 and DAS-59122-7. The levels of CP4 EPSPS produced in the combined trait product were similar to the levels produced in MON 88017. These results confirm that assessments based on protein levels of the single products are applicable to the combined trait product.

Efficacy of MON 89034 x TC1507 x MON 88017 x DAS-59122-7

Field efficacy studies were conducted in the US and Puerto Rico in 2006 and 2007 to compare the efficacy of the combined trait product to the single events against the corn rootworm complex and the lepidopteran insect complex in corn (Huckaba et al., 2008; Huckaba and Storer, 2008; Neese, 2008; Vaughn et al., 2008). Under high insect pressure, MON 89034 x TC1507 x MON 88017 x DAS-59122-7 had a greater level and consistency of CRW control compared to either single CRW- control event. No additional increase in the level of ECB, SWCB, or SCB control was seen for MON 89034 x TC1507 x MON 88017 x DAS-59122-7 compared to MON 89034 or TC1507 individually. The efficacy of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 against CEW

was similar to that of MON 89034. Control of FAW by MON 89034 x TC1507 x MON 88017 x DAS-59122-7 was excellent.

TABLE 1. Characerization reports for MON 89034 x TC1507 x MON 88017 x DAS-59112-7

Report	Report number
Taylor, J.P., J.R. Groat, and J.D. Masucci. 2007. Southern Blot Analyses to Confirm the Presence of MON 89034 and MON 88017 in the Combined Trait Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7	Monsanto Technical Report MSL0020682 MRID 47444903
Schafer, B.W., C.Q. Cai, and S.K. Embrey. 2008. Southern Blot Analysis to Confirm the Presence of TC1507 and DAS-59122-7 in the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7	Dow AgroSciences LLC Study 071179 MRID 47444904
Stillwell, L. and A. Silvanovich. 2007. Assessment of Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS Protein Levels in the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7 Produced in U.S. Field Trials During 2006	Monsanto Technical Report Number MSL0021070 MRID 47444906
Phillips, A. M. 2008. Cry34Ab1, Cry25Ab1, Cry1F and PAT Protein Levels in Hybrid Maize TC1507, DAS-59122-7, MON 89034 x TC1507 x MON 88017 x DAS-59122-7, and a Conventional Control from the Monsanto 2006 Production Plan 06-01-52-04	Dow AgroSciences LLC Study ID 061026.06 MRID 47444907
Levine, S.L., G. Mueller, and C. Jiang. 2008. Evaluation of the Potential for Interactions Among Cry Proteins Produced by MON 89034 x TC1507 x MON 88017 x DAS-59122-7 by Insect Bioassay	Monsanto Technical Report Number MSL0021104 MRID 47444910
MacRae, T. 2008. Evaluation of Potential for Interaction Between the *Bacillus thuringiensis* Proteins Cry3Bb1, Cry34Ab1, and Cry35Ab1	Monsanto Technical Report Number MSL0020554 MRID 47444909
Paradise, M.S., C. Jiang, and D.B. Carson. 2008. Evaluation of Potential Dietary Effects of Pollen from the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7 on the Ladybird Beetle, *Coleomegilla maculata* (Coleoptera:Coccinellidae)	Monsanto Technical Report MSL0021036 MRID 47444913
Vaughn, T.T., G.P. Head, J.A. Murphy, et al. 2008. Assessment of the Efficacy of Lepidopteran and Coleopteran-Protected Corn MON 89034 x TC1507 x MON 88017 x DAS-59122-7 Against Major Insect Pests in United States and Puerto Rico Field Trials During the 2006-2007 Seasons	Monsanto Technical Report RPN-07-262
Neese, P.A. 2008. Evaluation of the Combined Trait Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7 for Efficacy Against Lepidopteran Pests of Corn in the US in 2007	Dow AgroSciences LLC Corn Study 081029
Huckaba, R.M., P.A. Neese, S. Ferguson, et al. 2008. Evaluation of the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7 for Control of Western Corn Rootworm in the U.S. in 2007	Dow AgroSciences LLC Study 081030.

Data from pp. 18-19, MRID 47444901

TABLE 2. Summary of mean (range) protein levels[1] in representative tissues of MON 89034 x TC1507 x MON 88017 x DAS-59122-7, with MON 89034, EC1507, MON 888017, and DAS-59122-7, from 2006 US field trials					
Proteins/products	**OSL-1**	**OSR-1**	**OSWP-2**	**Pollen**	**Grain**
Cry1A.105					
Combined product[2]	140 (100-210)	73 (55-100)	19 (8.1-26)	14 (7.8-21)	4.3 (3.4-4.9)
MON 89034	130 (85--200)	70 (48-240)	18 (6.9-31)	7.1 (5.1-12)	2.8 (1.7-3.5)
Cry2Ab2					
Combined product[2]	220 (140-350)	54 (32-76)	29 (11-53)	0.81 (0.18-2.3)	5.7 (4.1-7.5)
MON 89034	210 (140-290)	50 (28-80)	29 (12-54)	0.76 (0.31-2.2)	5.6 (2.7-7.1)
Cry1F					
Combined product[2]	22.5 (15.4-31.1)	11.8 (5.85-14.7)	9.0 (6.29-12.2)	22.1 (14.3-32.2)	3.34 (2.12-7.43)
TC1507	22.8 (15.5-34.3)	11.0 (8.62-13.2)	7.69 (4.65-10.6)	20.3 (14.5-23.1)	3.15 (2.43-4.58)
PAT					
Combined product[2]	25.0 (18.1-34-9)	3.74 (2.39-7.34)	2.51 (1.56-4.45)	ND	§0.050 (ND-0.10)
TC1507	6.94 (4.70-9.94)	0.69 (0.20-0.90)	0.52 (0.26-0.81)	ND	ND
DAS-59122-7	16.9 (11.4-22.9)	2.39 (0.77-3.53)	1.58 (0.70-2.97)	ND	§0.049 (0.026-0.091)
Cry3Bb1					
Combined product[2]	310 (150-490)	160 (95-260)	48 (24-79)	15 (7.5-24)	18 (10-26)
MON 88017	330 (140-580)	140 (81-190)	40 (21-61)	12 (8.1-16)	20 (12-38)
CP4 EPSPS					
Combined product[2]	190 (110-270)	99 (71-110)	42 (29-52)	310 (190-480)	5.2 (3.5-7.1)
MON 88017	170 (82-240)	92 (58-120)	37 (21-51)	260 (170-340)	4.9 (3.3-7.4)
Cry34Ab1					
Combined product[2]	92.3 (71.9-141)	105 (84.2-150)	157 (113-194)	92.2 (77.5-117)	62.6 (47.8-94)
DAS-599122-7	92.3 (71.5-118)	105 (76.4-147)	168 (117-233)	84.4 (68-97.8)	66.5 (43.6-102)
Cry35Ab1					
Combined product[2]	69.4 (45.4-96.1)	49.5 (39.0-70.5)	33.6 (27.7-42.3)	0.09 (ND-0.53)	1.69 (1.24-2.31)
DAS-59122-7	70.2 (48.2-86)	52.9 (31.7-80.5)	37.1 (26.8-54.4)	§0.03 (ND-0.057)	1.86 (1.18-2.65)

Data from p. 23, MRID 47449001

[1]Values represent means and (range) in tissue as µg/g dry weight.

[2]Combined trait product MON 89034 x TC1507 x MON 88017 x DAS-59122-7

Tissues were collected at the following growth stages: OSL-1, OSR-1: V2-V4; OSWP-2 (forage): R4-R5; pollen R1 pollen shed; grain: R6 physiological maturity (Ritchie et al., 1997)

§Mean value was between the method LOD and LOQ

ND = Not detected

These data confirm that combining MON 89034 with TC1507, MON 88017, and DAS-59122-7 did not alter the level of lepidopteran control, and produced a greater level of rootworm control. The results were as expected across a broad geographical range and under various levels of insect pressure, demonstrating that the individual insect control efficacy of the single events was not altered when combined in MON 89034 x TC1507 x MON 88017 x DAS-59122-7.

Interaction Among the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, and Cry3435Ab1 Proteins

Studies using sensitive indicator insect species have demonstrated that the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, and Cry34Ab1/ Cry35Ab1 proteins act independently, with no synergism or antagonism. The lack of interaction permits previous studies performed for registration of the single events to support the safety assessment for the combined trait product.

To confirm the lack of interactions MON 89034 x TC1507 x MON 88017 x DAS-59122-7 was used in feeding studies with ECB as the sensitive target pest (Levine et al., 2008). The CRW-control proteins produced by the combined trait product were assessed in combination against SCRW (MacRae, 2008). Results of these studies demonstrated the lack of interactions among Cry proteins in the combined trait product. Additionally, exposure to pollen from the combined trait product at a dietary concentration of 50% w/w had no adverse effect on survival, development, or growth of the non-target insect *Coleomegilla maculata* (Paradise, et al., 2008).

Several studies have demonstrated a lack of interaction among the *Bt* proteins for subsets of the PIPs produced by MON 89034 x TC1507 x MON 88017 x DAS-59122-7 (Table 3). The results of these studies support the conclusion that there are no interactions among the various Cry proteins present in MON 89034 x TC1507 x MON 88017 x DAS-59122-7.

TABLE 3. Protein interaction studies for the Cry proteins expressed by MON 89034 x TC1507 x MON 88017 x DAS-59122-7

Product	Cry proteins	Interaction	Citation
DAS59122 x TC1507	Cry34/35Ab1, Cry1F	No	Herman and Storer, 2004
MON 89034	Cry1A.105, Cry2Ab2	No	MacRae et al., 2005
MON 89034 x MON 88017	Cry1A.105, Cry2Ab2, Cry3Bb1	No	MacRae et al., 2006
MON 89034 x TC1507 x MON 88017 x DAS-59122-7	Cry34/35Ab1, Cry3Bb1	No	MacRae et al., 2008; Levine et al., 2008
MON 89034 x TC1507 x MON 88017 x DAS-59122-7	Cry1A.105, Cry2Ab2, Cry1F	No	Levine et al., 2008
MON 89034 x TC1507 x MON 88017 x DAS-59122-7	Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34/35Ab1	No	Levine et al., 2008

Data from p. 26, MRID 47444901

Human Health Assessment

The human health and exposure assessments for each of the independent events, MON 89034, TC1507, MON 88017, and DAS 59122-7, were reviewed and accepted by various regulatory agencies (EPA 2001a, 2005a, 2005b, 2005d, 2008b; USDA, 2001, 2005a, 2005b; FDA, 2001, 2004, 2005, 2007). No significant concerns were raised for these products or the pesticidal and herbicide-tolerant proteins they produce. The prior assessments of the individual products, and the bridging analyses conducted on their combination, support the

conclusion that the events and proteins produced in the combined trait product will not have adverse effects on human health (EPA, 2004b).

Furthermore, there were no human health concerns for the commercial combined trait corn registered products TC1507 x DAS-59122-7 and MON 89034 x MON 88017. After reviewing these two smaller combined trait products, EPA determined that the mode of action for these proteins does not suggest an enhanced activity when they are combined in a single plant.

The safety of *Bt* proteins to humans is well established. Cry proteins have been used as components of *Bt* microbial pesticides for over 45 years and are widely considered to be nontoxic to humans or other mammals when tested individually or in combination (EPA, 1998, 2000a, 2000b, 2001c; Betz et al., 2000; USDA 2005a, 2005b; OECD, 2007). Their mode of action is specific to certain orders of insects, and there is a demonstrated lack of homology of Cry proteins to known human toxins or allergens. The mode of action for Cry proteins involves binding of the protein to Cry-specific receptors in the intestinal tissues of susceptible insect species. No known high-affinity binding receptors to Cry proteins are located on the intestinal tissues of mammalian species (Hofmann et al., 1988a, 1988b; Noteborn et al, 1994). Cry proteins administered to mammals at doses millions of times higher than target insects would ever encounter produced no adverse effects (Betz et al., 2000). Furthermore, the amino acid sequences of the Cry proteins in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 are not similar to anti-nutritional proteins or to any other known mammalian protein toxins (Hileman and Astwood, 1999a, 1999b; Hileman et al., 2001; McClain and Silvanovich, 2006a, 2006b; Meyer, 1999; Stelman, 2000, 2002; Cressman, 2003).

For *Bt* Cry proteins, EPA has determined that a high-dose acute test in mice is sufficient to confirm the absence of toxicity towards mammals (McClintock et al., 1995). Additionally, EPA has determined that the data generated for *Bt* proteins individually support the food safety determination in combined trait PIPs because the mode of action for *Bt* proteins does not suggest an enhanced activity in combination (EPA, 2005i, 2005j; 2008c). Therefore, the acute oral mouse toxicity studies for the individual Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, and Cry35Ab1 proteins are sufficient to confirm the lack of mammalian toxicity of the combined trait product. Based on a review of the data and information, EPA concluded that there is reasonable certainty that no harm will result from the exposure of the US population, including infants and children, to these Cry proteins (EPA, 2001a, 2001c, 2004a, 2005h, 2008a).

The CP4 EPSPS and PAT proteins produced by MON 89034 x TC1507 x MON 88017 x DAS-59122-7 are nontoxic, and have a mode of action dissimilar from each other and from *Bt* Cry proteins. CP4 EPSPS and PAT are classified as plant pesticide inert ingredients and are exempt from the requirement of tolerance per 40 CFR 174.523 and 174.522, respectively. The safety of potential residues of glufosinate and glyphosate herbicides on this PIP product should be considered separately from the safety of the CP4 EPSPS and PAT proteins.

The data and information provided lead to the conclusion that there is a reasonable certainty that no harm will result to the US population as a result of commercialization of or potential exposure to MON 89034 x TC1507 x MON 88017 x DAS-59122-7.

Potential Impact on Non-Target Organisms, Endangered Species, and the Environment

Laboratory bioassays using sensitive target insect species to generate dose-response curves demonstrated a lack of interaction among the four events and among the CRW-active proteins in the combined trait product (Levine et al., 2008; MacRae, 2008). This allows the environmental assessments conducted for the single events to be applied to the combined trait product (EPA, 2004b). Two years of field data across multiple sites support that the insecticidal efficacy against lepidopteran and corn rootworm pests is consistent with combining the independent activities of each of the single event products in an essentially additive manner (Huckaba et al., 2008; Neese, 2008; Vaughn et al., 2008; Head and Storer, 2008). Data from an array of non-target organism hazard studies conducted for each single event product further confirm the environmental safety of the combined trait product (Paradise et al., 2008). EPA (2004b) has concluded that unless there is an expectation of, or evidence for, interactions among insectidical proteins, non-target organism testing on individual proteins is sufficient to assess the safety of events with multiple *Bt* proteins, and testing of protein combinations is not required. Therefore, the previously defined activity spectra for each of the *Bt* proteins are applicable to the combined trait product.

Given the specificity of the *Bt* proteins produced by MON 89034 x TC1507 x MON 88017 x DAS-59122-7, species outside Lepidoptera and Coleoptera should not be affected. The potential hazard to non-target lepidopterans, coleopterans, and endangered species was previously assessed by EPA for each of the individual events and the Cry proteins they produce (EPA 2001a, 2005a, 2005b, 2005c, 2005d, 2005e, 2005f, 2005g, 2007, 2008b). None of these Cry proteins demonstrated unacceptable toxic effects on representative non-target organisms. EPA determined that no hazard exists to the environment from registering these individual events, and that no adverse effects on endangered or threatened species are expected. These conclusions were also confirmed in smaller combinations of these same events, TC1507 x DAS-59122-7 and MON 89034 x MON 88017.

Consistent with the single events, an assessment of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 concluded that no adverse effect is expected on endangered or threatened species or their critical habitats (Huesing and Levine, 2008). The insecticidal activity of Cry3Bb1 and Cry34/35Ab1 are specific to coleopterans. The only coleopteran with a terrestrial range overlapping an area of corn production is the American burying beetle (*Nicrophorus americanus*). The larvae and adults of this species feed exclusively on carrion, providing a negligible chance of exposure to *Bt* protein. Additionally, an analysis of the potential impact to threatened or endangered aquatic coleopterans from potential exposure to Cry3Bb1 and Cry34/35Ab1 in aquatic environments found negligible exposure.

Given the specificity of the Cry1A.105, Cry2Ab2, andCry1F proteins to Lepidoptera, it is unlikely that species in other orders would be directly affected. The only endangered lepidopteran species with a potential range overlap with corn production is the Karner blue butterfly (*Lycaeides melissa samuelis*). However, the habitat and feeding biology of the Karner blue preclude and exposure to pollen from MON 89034 x TC1507 x MON 88017 x DAS-59122-7. Additionally, a risk analysis indicates a ≥10-fold safety factor exists for MON 89034 x TC1507 x MON 88017 x DAS-59122-7 at distances ≥1.74 meters from the edge of a corn field.

Environmental Fate in Soil

Laboratory studies with the proteins produced in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 indicate that the *Bt* proteins are rapidly degraded in soil. Data from multi-year field soil studies with several *Bt*

corn and Bt cotton products have confirmed the lack of persistence of *Bt* proteins in soil. Therefore, the *Bt* Cry proteins in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 will not persist or accumulate in soils.

Benefits of Cultivation

Commercialization of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 may provide substantial economic benefits to growers by limiting yield losses from corn rootworm and lepidopteran pests, as well as from weed pressure. The combined trait product supports enhanced product durability/IRM value, lower refuge for lepidopteran and corn rootworm pests relative to single *Bt* corn products, grower convenience in broad spectrum pest management and IRM compliance, grain quality, and yield potential.

Study Authors' Conclusions

The study authors concluded that there is reasonable certainty that no harm will result to the US population or to the environment as a result of exposure to MON 89034 x TC1507 x MON 88017 x DAS-59122-7.

Reviewer's Conclusion

The safety of expected combined residues of the glufosinate and glyphosate herbicides should be addressed..

References

Beasley, K.A., H.M. Anderson, P.B. Wimberley, et al. 2002. Molecular Analysis of YieldGard Rootworm/Roundup Ready Corn Event MON 88017. Monsanto Technical Report MSL-17609. MRID 46181702.

Beckie, H.J. 2006. Herbicide-Resistant Weeds: Management Tactics and Practices. Weed Technology 20:793-814.

Bergstrom, C.T., M. Lo, and M. Lipsich. 2004. Ecological Theory Suggests That Antimicrobial Cycling Will Not Reduce Antimicrobial Resistance in Hospitals. PNAS 101:13285-13290. http://www.pnas.org/cgi/content/short/101/36/13285

Betz, F.S., B.G. Hammond, and R.L. Fuchs. 2000. Safety and Advantages of *Bacillus thuringiensis* Protected Plants to Control Insect Pests. Regul. Toxicol. Pharmacol. 32:156-173.

Brent, K.J. and D.W. Holloman. 2007. Fungicide Resistance in Crop Pathogens: How Can it be Managed? FRAC Monograph No. 1 (second, revised edition) Fungicide Resistance Action Committee http://www.frac.info/frac/publication/anhang/FRAC_Mono1_2007_100dpi.pdf

Cressman, R.F. 2003. Evaluation of Sequence Similarities of the Cry34Ab1, Cry35Ab1 and PAT Proteins to the Public Protein Sequence Datasets. MRID 64584701.

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EPA. 2000a. FIRRA Scientific Advisory Panel on Sets of Scientific Issues Being Considered by the Environmental Protection Agency Regarding: Bt Plant-Insecticides Risk and Benefits Assessments. October 18-20, 2000.

EPA. 2000b. Biopesticides Registration Action Document. Preliminary Risks and Benefits Sections. *Bacillus thuringiensis* Plant Pesticides. http://www.epa.gov/scipoly/sap/meetings/2000/october/brad1_execsum_overview.pdf

EPA. 2001a. *Bacillus thuringiensis* subspecies Cry1F Protein and the Genetic Material Necessary for its Production (Plasmid Insert PHI 8999) in (004681) Corn Fact Sheet. US Environmental Protection Agency. http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm

EPA. 2001b. *Bacillus thuringiensis* Cry1F Protein and the Genetic Material Necessary for its Production in Corn. Exemption from the Requirement of a Tolerance. US Environmental Protection Agency. Fed. Reg. 66(109):30321-30325.

EPA. 2001c. *Bacillus thuringiensis* Cry3Bb1 and Cry2Ab2 Protein and the Genetic Material Necessary for its Production in Corn and Cotton. Exemption from the Requirement of a Tolerance; Final Rule. US Environmental Protection Agency. Fed. Reg. 66(92):24061-24066.

EPA. 2004a. *Bacillus thuringiensis* Cry3Bb1. Exemption from the Requirement of a Tolerance. Final Rule. US Environmental Protection Agency. Fed. Reg. 66(62):16089-16814.

EPA. 2004b. FIFRA Scientific Advisory Panel Meeting. SAP Report 2004-05. Product Management for *Bacillus thuringiensis* (Bt) Cotton Products. US Environmental Protection Agency, Washington, DC. http://www.epa.gov.scipoly/sap/meetings/2004/june/final1a.pdf

EPA. 2005a. *Bacillus thuringiensis* subspecies Cry1F Protein and the Genetic Material Necessary for its Production (Plasmid Insert GHI 8999) in (004681) Corn Fact Sheet. US Environmental Protection Agency. http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm

EPA. 2005b. *Bacillus thuringiensis* subspecies Cry3Bb1 Protein and the Genetic Material Necessary for its Production (Vector ZMIR13L) in Event MON863 Corn (006484) Fact Sheet. US Environmental Protection Agency. http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm

EPA. 2005c. US Environmental Protection Agency Office of Pesticide Programs Biopesticide Registration Action Document. *Bacillus thuringiensis* Cry1F Corn. US Environmental Protection Agency. http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm

EPA. 2005d. *Bacillus thuringiensis* Cry34Ab1 and Cry35Ab1 Proteins and the Genetic Material Necessary for Their Production (Plasmid Insert PHP 17662) In Event DAS-59122-7 Corn (006490) Fact Sheet. US Environmental Protection Agency. http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm

EPA. 2005e. Biopesticides Registration Action Document *Bacillus thuringiensis* Cry34Ab1 and Cry35Ab1 and the Genetic Material Necessary for Their Production in Event DAS-59122-7 Corn. US Environmental Protection Agency. http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm

EPA. 2005f. *Bacillus thuringiensis* Cry3Bb1 Protein and the Genetic Material Necessary for it Production (Vector ZMIR39) in Event MON 88017 Corn (OECD Unique Identifier: MON-88017-3) (006502) and *Bacillus thuringiensis* Cry1Ab Delta-Endotoxin and the Genetic Material Necessary for its Production in Corn (006430) Fact Sheet. http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm

EPA. 2005g. *Bacillus thuringiensis* Cry3Bb1 Protein and the Genetic Material Necessary for its Production (Vector ZMIR13L) in Event MIR603 Corn (006484). Biopesticides Registration Action Document (BRAD). US Environmental Protection Agency. http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm

EPA. 2005h. *Bacillus thuringiensis* Cry34Ab1 and Cry35Ab1 Proteins and the Genetic Material Necessary for Their Production in Corn. Exemption from the Requirement of a Tolerance. Final Rule. US Environmental Protection Agency. Fed. Reg.70(182):55254-55250.

EPA. 2005i. US Environmental Protection Agency Office of Pesticide Programs. Biopesticides Registration Action Document (BRAD). (*Bacillus thuringiensis* Cry1F (Synpro and Cry1Ac (Synpro) Construct 281/3006 Insecticidal Crystal Proteins as Expressed in Cotton) (Chemical PC Codes 006512 and 006513, respectively). http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm

EPA. 2005j. US Environmental Protection Agency Office of Pesticide Programs. *Bacillus thuringiensis* Cry34Ab1 and Cry35Ab1 Proteins and the Genetic Material Necessary for Their Production (Plasmid Insert PHP 17662) in Event DAS-59122-7 Corn & *Bacillus thuringiensis* Cry1F Protein and the Genetic Material Necessary for its Production (Plasmid Insert PHI8999) in Event TC1507 Corn (006490, 006481) Fact Sheet. http://www.epa.gov/oppbppd1/biopesticides/ingredients/factsheets/factsheet_006481-006490.htm

EPA. 2007. *Bacillus thuringiensis* Cry3Bb1 Protein and the Genetic Material Necessary for its Production (Vector ZMIR13L) in Event MON863 Corn (006484) Biopesticides Registration Action Document (BRAD). US Environmental Protection Agency. http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm

EPA. 2008a. Exemptions from the Requirement of a Tolerance for the *Bacillus thuringiensis* Cry1A.105 and Cry2Ab2 Proteins Were Established in Corn, June 10, 2008.

EPA. 2008b. MON 89034. EPA Registration 534-575, June 10, 2008.

EPA. 2008c. MON89034 x MON 88017. EPA Registration 524-576, June 10, 2008.

FDA. 2001. US Food and Drug Administration. BNF 73. TC1507. Downloaded from http://www.cfsan.fda.gov/~lrd/biocon.html

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FDA. 2004. US Food and Drug Administration. BNF 81. DAS-59122-7. Downloaded from http://www.cfsan.fda.gov/~lrd/biocon.html

FDA. 2005. US Food and Drug Administration. BNF 97. MON 88017. Downloaded from http://www.cfsan.fda.gov/~lrd/biocon.html

FDA. 2007. US Food and Drug Administration. BNF 107. MON 89034. Downloaded from http://www.cfsan.fda.gov/~lrd/biocon.html

Gressel, J. and L.A. Segel. 1990. Modeling the Effectiveness of Herbicide Rotations and Mixtures as Strategies to Delay or Preclude Resistance. Weed Technology 4:186-198.

Gustafson, D.I. and G.P. Head. 2008. Modeling the Impact of a Five-Percent Structured Refuge on the Evolution of Corn Rootworm Resistance to MON 89034 x TC1507. MON 88017 x DAS-59122-7. Monsanto Technical Report MSL-0021189.

Head, G. and N. Storer, 2008. Insect Resistance Management Plan for MON 89034 x TC1507 x MON 88017 x DAS-59122-7. Monsanto Technical Report MSL0021285.

Hileman, R.E. and J.D. Astwood. 1999a. Bioinformatics Analysis of *B.t.* Protein 11098 and *B.t.* Progein 11231 Sequences Utilizing Toxin and Public Domain Genetic Databases. MSL-15870. MRID 44904308.

Hileman, R.E. and J.D. Astwood. 1999b. Bioinformatics Analysis of *B.t.* Protein 11098 and *B.t.* Progein 11231 Sequences Utilizing an Allergen Database. MLS-15873. MRID 44904309.

Hileman, R.E., E.A. Rice, R.E. Goodman, et al. 2001. Bioinformatics Evaluation of the Cry3Bb1 Protein Produced in MON 863 Utilizing Allergen, Toxin and Public Domain Protein Databases. Monsanto Technical Report MSL-17140. MRID 45424008.

Hofmann, C., P. Luthy, R. Hutter, et al. 1988a. Binding of the Delta-Endotoxin from *Bacillus thuringiensis* to Brush-Border Membrane Vesicles of the Cabbage Butterfly (*Pieris brassicae*). Eur. J. Biochem. 173:85-91.

Hofmann, C., H. Vanderbruggen, H. Hofte, et al. 1988b. Specificity of *Bacillus thuringiensis* Delta-Endotoxins is Correlated with the Presence of High Affinity Binding Sites in the Brush-Border Membrane of Target Insect Midguts. Proc. Natl. Acad. Sci. USA 85:7844-7848.

Hondred, D. 1998. Characterization of Gene Inserts – *Bacillus thuringiensis* var. *aizawai* Cry1F Insect Control Protein as Expressed in Maize. Pioneer Hi-Bred International Internal Report. MRID 44714802.

Hondred, D. 1999. Characterization of Inserted Genes in Cry1F Maize Line 1507. Pioneer Hi-Bred International Internal Report. MRID 45020102.

Huckaba, R.M. and N.P. Storer. 2008. Field Measures of Western Corn Rootworm (Coleoptera:Chrysomelidae) Mortality Caused by Cry34/Cry35Ab1 and Cry3Bb1 Proteins in the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7. Dow AgroSciences LLC Study 081028.

Huckaba, R.M., P.A. Neese, S. Ferguson, et al. 2008. Evaluation of the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7 for Control of Western Corn Rootworm in the U.S. in 2007. Dow AgroSciences LLC Study 081030.

Huesling, J.E. and S.L. Levine. 2008. Endangered Species Impact Assessment for the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7. Monsanto Technical Report MSl0021268.

Levine, S.L., G. Mueller, and C. Jiang. 2008. Evaluation of the Potential for Interactions Among Cry Proteins Produced by MON 89034 x TC1507 x MON 88017 x DAS-59122-7 by Insect Bioassay. Monsanto Technical Report Number MSL0021104.

Li, Y.J. and L. Zhou. 2008. Comparative Binding of the *Bacillus thuringiensis* Cry3Bb1 and Cry34/35Ab1 Proteins to Western Corn Rootworm (*Diabrotica virgifera*) Brush Border Membranes. Monsanto Technical Report MS0021104.

Locke, M. 2003. Detailed Characterization of DNA Inserted into Transgenic Corn Events DAS-45216-6 and DAS-59122-7. Unpublished Report of Pioneer Hi-Bred International, Inc. PHI-2002-041.

MacRae, T. 2008. Evaluation of Potential for Interaction Between the *Bacillus thuringiensis* Proteins Cry3Bb1, Cry34Ab1, and Cry35Ab1. Monsanto Technical Report Number MSL0020554.

McClain, J.S. and A. Silvanovich. 2006a. Bioinformatics Evaluation of the Cry1A.105 Protein Utilizing the AD6, TOXIN5 and ALLPEPTIDES Databases. Monsanto Technical Report MSL-20351. MRID 46951410.

McClain, J.S. and A. Silvanovich. 2006b. Bioinformatics Evaluation of the Cry2Ab2 Protein Utilizing the AD6, TOXIN5 and ALLPEPTIDES Databases. Monsanto Technical Report MSL-20307. MRID 46951411.

McClintock, et al. 1995. Reference not provided.

Meyer, T. 1999. Comparison of Amino Acid Sequence Similarity of Cry1F and PAT Proteins to Known Allergen Proteins. MRID 4497101.

NATAP Reports. 1998. National AIDS Treatment Advocacy Project, NATAP Resistance Supplement. http://www.natap.org/1998/98reports/natap_reports3.10.htm

Neese, P.A. 2008. Evaluation of the Combined Trait Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7 for Efficacy Against Lepidopteran Pests of Corn in the US in 2007. Dow AgroSciences LLC Corn Study 081029.

Noteborn, H.P.J.M., M.E. Rienenmann-Ploum, J.H.J. van den Berg, et al. 1994. Consuming Transgenic Food Crops: The Toxicological and Safety Aspects of Tomato Expressing Cry1Ab and NPTII. ECB6: Proceedings of the 6th European Congress on Biotechnology, Elsevier, Amsterdam.

Paradise, M.S., C. Jiang, and D.B. Carson. 2008. Evaluation of Potential Dietary Effects of Pollen from the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7 on the Ladybird Beetle, *Coleomegilla maculata* (Coleoptera:Coccinellidae). Monsanto Technical Report MSL0021036.

Phillips, A. M. 2008. Cry34Ab1, Cry25Ab1, Cry1F and PAT Protein Levels in Hybrid Maize TC1507, DAS-59122-7, MON 89034 x TC1507 x MON 88017 x DAS-59122-7, and a Conventional Control from the Monsanto 2006 Production Plan 06-01-52-04. Dow AgroSciences LLC Study ID 061026.06.

Rice, J.F., B.J. Wolff, J.R. Groat, et al. 2006. Molecular Analysis of Corn MON 89034 Amended Report. Monsanto Technical Report MSL-20311. MRID 46951402.

Schafer, B.W., C.Q. Cai, and S.K. Embrey. 2008. Southern Blot Analysis to Confirm the Presence of TC1507 and DAS-59122-7 in the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7. Dow AgroSciences LLC Study 071179.

Schlenz, M.L., J. M. Babcock, and N.P. Storer. 2008. Response of Cry1F-Resistant and Susceptible European Corn Borer and Fall Armyworm Colonies to Cry1A.105 and Cry2Ab2. Dow AgroSciences LLC Study 08036.

Simone, P.M. and S.W. Dooley. 1994. Multidrug-Resistant Tuberculosis. Centers for Disease Control (CDC) Division of Tuberculosis Elimination. http://www.cdc.gov/tb/pubs/mdrtb/default.htm

Stelman, S.J. 2000. Comparison of the Amino Acid Sequence of the *Bacillus thuringiensis* Strain PS149B1 13.6kDa and 43.8kDa Insecticidal Crystal Proteins to Known Protein Allergens. MRID 45242205.

Stelman, S.J. 2002. Comparison of the Amino Acid Sequence of the *Bacillus thuringiensis* var. *aizawai* Cry1F Insect Crystal Protein as Expressed in *Zea mays* Event 1507 to Assess Homology to Known Protein Toxins. Unpublished Report of Dow AgroSciences LLC.

Stillwell, L. and A. Silvanovich. 2007. Assessment of Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS Protein Levels in the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7 Produced in U.S. Field Trials During 2006. Monsanto Technical Report Number MSL0021070.

Storer, N. 2008. Simulation Modeling of Corn Rootworm Adaptation to Pyramided Bt Corn Plant Incorporated Protectants MON 88017 and DAS-59122-7. Dow AgroSciences LLC Study 081024.

Taylor, J.P., J.R. Groat, and J.D. Masucci. 2007. Southern Blot Analyses to Confirm the Presence of MON 89034 and MON 88017 in the Combined Trait Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7. Monsanto Technical Report MSL0020682.

Vaughn, T.T., G.P. Head, J.A. Murphy, et al. 2008. Assessment of the Efficacy of Lepidopteran and Coleopteran-Protected Corn MON 89034 x TC1507 x MON 88017 x DAS-59122-7 Against Major Insect Pests in United States and Puerto Rico Field Trials During the 2006-2007 Seasons. Monsanto Technical Report RPN-07-262.

Weber, N. 2004. Detailed Characterization of a Stacked Hybrid of Transgenic Corn Lines DAS-01507-1 and DAS-59122-7. Unpublished Report of Pioneer Hi-Bred International, Inc. PHI-2003-049.

WHO. 2001. Use of Antimalarial Drugs, Report of an Informal Consultation. 2. Combination Therapy. http://www.rbm/int/cmc_upload/0/000/014/923/am_2.htm

WHO/TDR. 2000. World Health Organization Special Programme for Research and Training in Tropical Diseases. Methods for Treatment, Malaria: Drug Resistance and Policies. TDR Fifteenth Programme Report, Progress 1999-2000. http://www.who.int/tdr/research/progress9900/methods/default.htm

Wrubel, R.P. and J. Gressel. 1995. Are Herbicide Mixtures Useful for Delaying the Rapid Evolution of Resistance? A Case Study. Weed. Technology 8:635-648.

Zhuang, M. 2008. Differentiating the Binding Sites of Two Groups of Coleopteran-Active *Bacillus thuringiensis* Cry Proteins, Cry34Ab1/Cry3Ab1 and Cry3Bb1. Dow AgroSciences LLC Study 081027.

DATA EVALUATION RECORD

Primary Reviewer: H. Tim Borges, Ph.D., MT(ASCP), DABT, contract reviewer, ORNL
EPA Secondary Reviewer: John L. Kough, Ph.D., Biologist

STUDY TYPE:	Southern Blot Analysis
MRID NO:	47444902
DP BARCODE:	355689
TEST MATERIAL:	DNA from plants expressing *Bacillus thuringiensis* toxins
PROJECT REPORT NO:	MSL0021265
SPONSOR FACILITY:	Monsanto Company 800 N. Lindbergh Blvd. St. Louis, MO 63167
TITLE OF REPORT:	Summary of Southern blot analyses to confirm the presence of MON 89034, TC1507, MON 88017, and DAS-59122-7 in the combined trait corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7
AUTHOR:	Rice, JF
STUDY COMPLETED:	April 11, 2008
CONCLUSION:	The results of Southern blot analyses showed that the hybridization patterns of Southern blot analysis for the combined train corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 were identical to the respective single products MON 89034, TC1507, MON 88017, and DAS-59122-7.
CLASSIFICATION:	Supplemental. Summary document only
GOOD LABORATORY PRACTICE:	Not applicable

A. **STUDY PURPOSE:** This report is a summary of the Southern blot results presented in MRIDs 4744903 and 4744904.

B. **RESULTS:** The results of Southern blot analyses showed that the hybridization patterns of Southern blot analyses for the combined train corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 were identical to the respective single products MON 89034, TC1507, MON 88017, and DAS-59122-7 as described in MRIDs 4744903 and 4744904.

DATA EVALUATION RECORD

Primary Reviewer: H. Tim Borges, Ph.D., MT(ASCP), DABT, Contract Reviewer, ORNL
EPA Reviewer: John L. Kough, Ph.D., Biologist

STUDY TYPE:	Southern Blot Analysis
MRID NO:	47444903
DP BARCODE:	355689
TEST MATERIAL:	DNA from plants expressing *Bacillus thuringiensis* toxins
PROJECT REPORT NO:	07-01-52-10, MSL 0020682
TESTING FACILITY:	Monsanto Company Product Characterization Center Biotechnology Regulatory Sciences 800 North Lindbergh Blvd. St. Louis, MO 63167
TITLE OF REPORT:	Southern blot analysis to confirm the presence of MON 89034 and MON 88017 in the combined trait corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7
AUTHOR:	Taylor, JP; Groat, JR; Masucci, JD
STUDY COMPLETED:	November 14, 2007
CONCLUSION:	Southern blot analysis confirmed in the combined trait corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 the presence of sequences identical to sequences derived from MON 89034 and MON 88017.
CLASSIFICATION:	Acceptable
GOOD LABORATORY PRACTICE:	Yes

A. **STUDY PURPOSE:** The study was done to confirm the presence of the MON 89034 and MON 88017 in the combined trait corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 by Southern blot analyses. MON 89034 produces the proteins Cry2Ab2 and Cry1A.105 derived from *Bacillus thuringiensis* (*kurstaki* strain) to selectively control larvae of the European corn borer (*Ostrinia nubilalis*) and other lepidopteran insect pests. MON 88017 produces a modified *Bacillus thuringiensis* (*kumamotoensis* strain) Cry3Bb1 protein to protect against corn rootworm larvae. In addition, MON 88017 produces a 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* strain CP4 which confers tolerance to glyphosate, the active ingredient in Roundup® agricultural herbicides.

B. **MATERIALS AND METHODS:** DNA was extracted from MON 89034 × TC1507 × MON 88017 × DAS-59122-7 corn and control corn DNA sequences obtained from MON 89034 and MON 88017 varieties. These were compared to reference plasmid sequences in PV-ZMIR39 (produces *cp4 epsps* and *cry3Bb1* coding sequences) and PV-ZMIR245 (produces *cry1A.*105 and a portion of *cry2Ab2* coding sequences) used to develop MON 88017 and MON 89034, respectively. The identity of the test and control substances was confirmed by event-specific PCR.

Digested DNA samples were electrophoresed through 0.8% agarose gels. The DNA samples were transferred to membranes suitable for Southern blot analysis and DNA ^{32}P-probes specific for the *cp4 epsps, cry3Bb, cry1A.*105, *cry2Ab2 1* were prepared. The Southern blot

membranes containing the bound DNA were incubated with the probes and visualized by exposure to X-ray film.

C. **RESULTS:** When examined simultaneously with the MON 89034 specific probes *cry1A.105* and *cry2Ab2*, plasmid PV-ZMIR245 DNA produced the expected bands at ~7.2 kb and ~8.2 kb in MON 89034 × TC1507 × MON 88017 × DAS-59122-7. Conventional corn DNA showed no detectable hybridization bands. Likewise, when examined with the MON 88017 specific probes TX-*CTP2*/CS-*cp4 epsps* and CS-*cry3Bb1*, plasmid PV-ZMIR39 DNA produced the expected bands at ~7.4 and 5.5 kb in the combined trait product while conventional corn DNA showed no detectable hybridization bands. Southern blot analyses confirmed in the combined trait corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 the presence of sequences identical to sequences in MON 89034 and MON 88017. The agarose gel autoradiographs and plasmid maps are found in the attached appendix. No molecular weight standards were indicated on the autoradiographs directly but were noted to the side as arrows.

3/3/09 OPP Review of SmartStax

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* The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DATA EVALUATION RECORD

Primary Reviewer: H. Tim Borges, Ph.D., MT (ASCP), DABT, Oak Ridge National Labs
EPA Secondary Reviewer: John L. Kough, Ph.D., Biologist

STUDY TYPE:	Southern Blot Analysis
MRID NO:	47444904
DP BARCODE:	355689
TEST MATERIAL:	DNA from corn with *Bacillus thuringiensis* proteins
PROJECT REPORT NO:	071179
TESTING FACILITY:	Reg.Sciences and Government Affairs – Indianapolis Lab Dow AgroSciences, LLC 9330 Zionsville Road Indianapolis, Indiana 46268-1054
TITLE OF REPORT:	Southern blot analysis to confirm the presence of TC1507 and DAS-59122-7 in the combined trait corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7
AUTHOR:	BW Schafer, CQ Cai, SK Embrey
STUDY COMPLETED:	March 26, 2008
CONCLUSION:	Southern blot analysis confirmed the presence of TC1507 and DAS-59122-7 in the combined trait corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7. Hybridization patterns for the combined trait product were identical to those of the parental lines with *cry*1F, *cry*34Ab1, *cry*35Ab1, and the *pat* gene probes indicating that the TC1507 and DAS-59122-7 insertions were unaffected by combining with MON 89034 and MON 88017 through conventional breeding.
CLASSIFICATION:	Acceptable
GOOD LABORATORY PRACTICE:	Yes

A. **STUDY PURPOSE:** The study was done to confirm the presence of the TC1507 and DAS-59122-7 inserts in the combined trait corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 by Southern blot analyses. TC1507 produces the *Bacillus thuringiensis* var *aizawai* Cry1F protein to selectively control larvae of the European corn borer (*Ostrinia nubilalis*) and other lepidopteran insect pests. DAS59122-7 produces the *Bacillus thuringiensis* strain PS149B1, Cry34Ab1, and Cry 35Ab1 proteins to protect against coleopteran pests such as corn rootworm. In addition, both TC1507 and DAS-59122-7 produce the phosphinothricin acetyl transferase (PAT) protein from *Streptomyces viridochromogenes*, to confer tolerance to glufosinate-ammonium, the active ingredient in Liberty® herbicide.

B. **MATERIALS AND METHODS:** DNA was prepared from grain of the combined trait corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 and control substances were prepared from grains of the single events TC1507 and DAS-59122-7. A 1 kb DNA molecular weight marker containing a mixture of DNA segments served as a size reference for the agarose gel electrophoresis and Southern blot analyses. Suitable reference plasmid controls were used for the *pat* and *cry*1F sequences in TC1507 (PHP8999) and the *cry*34Ab1, *cry*35Ab1, and *pat*

DNA sequences in DAS-59122-7 (PHP17661). The DNA probes specific for the *cry*1F, *cry*34Ab1, *cry*35Ab1, and the *pat* genes were produced by PCR.

The DNA samples (10 µg) from each of the samples and the conventional control were digested by the addition of three units of restriction enzyme/µg. The restriction enzymes used for the digests included *Hind* III, *Sac* I, *Nde* I, and *Nhe* I. The digested samples were then precipitated and resuspended in 1x Blue Juice for gel loading. The samples and the molecular weight marker were electrophoresed through 0.8% agarose gels for approximately 18 hours and stained with ethidium bromide and the DNA visualized under UV light. Southern blots analyses were done according to the method of Memelink, *et al* (1994).

DNA probes specific for the *cry*1F, *cry*34Ab1, *cry*35Ab1, and the *pat* genes and the 1 kb molecular weight marker were radioactively labeled with ^{32}P-dCTP (deoxycytidine triphosphate) and the activity of the labeled probes determined by LSC. The Southern blot membranes containing the bound DNA were incubated with approximately two million counts of the labeled gene specific probes/mL and allowed to hybridize overnight. The membranes were then rinsed and washed and the membranes exposed to X-ray film for two to 18 days. The X-ray film was then developed and analyzed.

C. **RESULTS:** Following *Hind* III hybridization, two fragments consistent with *cry*1F, one at 3890 bp and one at ~4100 bp, were observed in both TC 1507 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7. *Hind* III digestion also produced three bands consistent with the *pat* gene at 2170 bp with border fragments of >1090 bp and >2715 bp. The T-DNA of PHP17662 also hybridized the *pat* gene producing a single band at 6963 bp in DAS-59122-7. All four *pat* bands were produced in MON 89034 × TC1507 × MON 88017 × DAS-59122-7.

Sac I digestion produced a border fragment of ~3400 bp consistent with *cry*34Ab1 and an internal band of 1941 bp consistent with *cry*35Ab1 in DAS-59122-7 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7. *Sac* I digestion also hybridized the *pat* gene producing an internal fragment of 2108 bp and border fragments of >1096 bp and >6762 bp in TC1507 and 1855 bp in DAS-59122-7. All four bands were observed in MON 89034 × TC1507 × MON 88017 × DAS-59122-7.

Nhe I digestion produced an internal fragment when hybridized with *cry*34Ab1 of 2731 bp and *Nde* I generated a border fragment of >5384 bp with *cry35*Ab1 in DAS-59122-7. Both of these fragments were observed in MON 89034 × TC1507 × MON 88017 × DAS-59122-7.

Southern blot analysis confirmed the presence of TC1507 and DAS-59122-7 in the combined trait corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7. Hybridization patterns for the combined trait product were identical to those of the parental lines with *cry*1F, *cry*34Ab1, *cry*35Ab1, and the *pat* gene probes indicating that the TC1507 and DAS-59122-7 insertions were unaffected by combining with MON 89034 and MON 88017 through conventional breeding.

The plasmid maps, schematic diagrams for the southern blots and copies of the autoradiographs themselves are attached in appendix 1.

REVIEWER'S COMMENT: The autoradiography signals of gel bands at 4100bp for the *pat* gene positive probe in a *HinD*III were too low to be visible. The autoradiography signals of gel bands at 10,000bp for the *pat* gene positive probe in a *Sac*I were also too low to be visible.

In the *Sac*I digest gel of the *cry34Ab1* trait, the plasmid digest + conventional corn lane, the plasmid band is not the right size for the indicated *Sac*I cut sites from the plasmid map of PHP17662. The *cry34Ab1* band should be about 13,000 bp based on the *Sac*I sites at 40323 and 2979. Perhaps there was a mistake in referring to the map of plasmid 17662 and the gels of plasmid 17661.

REFERENCES: Memelink, J; Swords, K; Hoge, HJ (1994). Southern, northern, and western blot Analysis. Plant Mol. Biol. Manual F1:1-23

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* The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DATA EVALUATION RECORD

Primary Reviewer: Eric B. Lewis, M.S., Contract Reviewer, Oak Ridge National Laboratories
EPA Secondary Reviewer: JohnL. Kough, Ph.D., Biologist

STUDY TYPE:	Assessment of Protein Levels (Nonguideline)
MRID NO:	47444905
DP BARCODE:	DP355690
DECISION NO:	394799
SUBMISSION NO:	830991
TEST MATERIAL:	MON 89034 x TC1507 x MON 88017 x DAS-59122-7 (a.i., Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1)
STUDY NO:	MSL0021266
SPONSOR:	Monsanto Company, 800 North Lindbergh Blvd., St. Louis, MO 63167
TESTING FACILITY:	Not applicable
TITLE OF REPORT:	Summary of Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, CP4 EPSPS, Cry34Ab1, Cry35Ab1, and PAT Protein Levels in the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7 Produced in U.S. Field Trials During 2006.
AUTHORS:	Murphy, J.A., and S. McClain
STUDY COMPLETED:	April 7, 2008
CONFIDENTIALITY CLAIMS:	None.
GOOD LABORATORY PRACTICE:	A signed and dated compliance statement was provided. The study does not meet the requirements of 40 CFR Part 160, as it is not a study *per se*, but summarizes data from other reports.
STUDY SUMMARY:	Levels of the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, CP4 EPSPS, Cry34Ab1, Cry35Ab1, and PAT proteins were determined by validated enzyme linked immunosorbent assay in corn tissues collected from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 produced at five US field sites in 2006. In the same manner, levels of the Cry1Ab.105 and Cry2Ab2 proteins in MON 89034, the Cry1F and PAT proteins in TC1507, the Cry3Bb1 and CP4 EPSPS proteins in MON 88017, and the Cry34Ab1, Cry35Ab1, and PAT proteins in DAS-59122-7 were determined in tissues of these corns grown alongside MON

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89034 x TC1507 x MON 88017 x DAS-59122-7. The mean levels of each protein found in tissues from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 and the respective single trait corns support the conclusion that the levels of Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, and Cry35Ab1 proteins produced in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 are comparable to the corresponding levels produced by the appropriate single product corns. The levels of PAT in the combined trait product were as expected for the combined expression in TC1507 and DAS-59122-7, and the level of CP4 EPSPS produced in the combined trait product were similar to the level produced in MON 88017.

CLASSIFICATION: **Supplemental. Summary only.**

Introduction

MON 89034 x TC1507 x MON 88017 x DAS-59122-7 is a combined trait corn product that confers insect resistance by expressing the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, and Cry35Ab1 proteins, and herbicide tolerance by expressing the CP4 EPSPS and PAT proteins. Levels of the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, CP4 EPSPS, Cry34Ab1, Cry35Ab1, and PAT proteins were determined by validated enzyme linked immunosorbent assay (ELISA) in corn tissues collected from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 produced at five US field sites in 2006. In the same manner, levels of the Cry1Ab.105 and Cry2Ab2 proteins in MON 89034, the Cry1F and PAT proteins in TC1507, the Cry3Bb1 and CP4 EPSPS proteins in MON 88017, and the Cry34Ab1, Cry35Ab1, and PAT proteins in DAS-59122-7 were determined in tissues of these corns grown alongside MON 89034 x TC1507 x MON 88017 x DAS-59122-7.

Test Methods

Tissue samples included in the ELISA were: over season leaf (OSL 1-3), over season root (OSR 1-3), over season whole plant (OSWP 1-3), pollen, and grain. The over season samples were collected at three different growth stages (Ritchie, et al., 1997): for leaves, OSL-1 at stages V2-V4, OSL-2 at V8-V10, and OSL-3 at R1; for roots, OSR-1 at stages V2-V4, OSR-2 at V5-V6, and OSR-3 at R1; and for whole plants, OSWP-1 at stages V10-V12, OSWP-2 at R4-R5, and OSWP-3 at R6. Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS levels for all tissue types were calculated on a µg/g fresh weight basis. Moisture content was measured for all tissue types, and the protein levels were then calculated on a µg/g dry weight basis. Cry1F, Cry34Ab1, Cry35Ab1, and PAT protein levels for all tissues were reported on a dry weight basis, since the tissues were lyophilized prior to analysis.

Results Summary and Study Authors' Conclusion

The mean levels of each protein in tissues from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 and the respective single trait corns are summarized in Tables 1-5. The results support the conclusion that the levels of Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, and Cry35Ab1

proteins produced in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 are comparable to the corresponding levels produced by the appropriate single product corns. The levels of PAT in the combined trait product were as expected for the combined expression in TC1507 and DAS-59122-7, and levels of CP4 EPSPS produced in the combined trait product were similar to the levels produced in MON 88017.

Table 1. Cry1A.105 and Cry2Ab2 Protein Levels in MON 89034 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7

	Cry1A.105		Cry2Ab2	
	MON 89034	MON 89034× TC1507 x MON 88017× DAS-59122-7	MON 89034	MON 89034× TC3507 x MON 88017× DAS-59322-7
Tissue Type[1]	Mean (Range) (µg/g dwt)[2]	Mean (Range) (µg/g dwt)	Mean (Range) (µg/g dwt)	Mean (Range) (µg/g dwt)
OSWP-1	43 (21 – 66)	53 (32 – 86)	54 (21 – 130)	53 (21 – 80)
OSWP-2	18 (6.9 – 31)	19 (8.1 – 26)	29 (12 - 54)	29 (11 – 53)
OSWP-3	8.8 (3.9 – 22)	11 (3.8 – 21)	15 (3.6 – 38)	16 (4.5 – 41)
OSR-1	70 (48 – 240)	73 (55 – 100)	50 (28 – 80)	54 (32 – 76)
OSR-2	47 (31 – 81)	47 (32 – 64)	69 (22 – 120)	58 (26 – 120)
OSR-3	20 (15 – 34)	21 (11 – 30)	16 (4.0 – 40)	18 (4.1 – 49)
OSL-1	130 (85 – 200)	140 (100 – 210)	210 (140 – 290)	220 (140 – 350)
OSL-2	63 (44 – 89)	79 (47 – 120)	140 (76 – 330)	140 (60 – 200)
OSL-3	77 (39 – 140)	80 (53 – 120)	180 (120 – 240)	180 (140 – 220)
Pollen	7.1 (5.1 – 12)	14 (7.8 – 21)	0.76 (0.31 - 2.2)	0.81 (0.18 - 2.3)
Grain	2.8 (1.7 - 3.5)	4.3 (3.4 - 4.9)	5.6 (2.7 - 7.1)	5.7 (4.1 - 7.5)

1. Tissues were collected at the following growth stages (Ritchie, et al., 1997)
   a. OSL-1: V2 – V4; OSL-2: V8-V10; OSL-3: R1
   b. OSR-1: V2 – V4; OSR-2: V5-V6; OSR-3: R1
   c. OSWP-1: V10 – V12; OSWP-2: R4 – R5; OSWP-3: R6
   d. Pollen: during pollen shed (R1) e. Grain: R6/maturity
2. Protein levels are expressed on a dry weight basis (dwt)

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Table 2. Cry1F Protein Levels in TC1507 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7

	Cry1F	
	TC1507	MON 89034× TC1507 x MON 88017× DAS-59122-7
Tissue Type	Mean (Range) (µg/g dwt)[2]	Mean (Range) (µg/g dwt)
OSWP-1	10.0 (6.85 – 13.0)	11.2 (7.53 – 15.8)
OSWP-2	7.69 (4.65 – 10.6)	9.00 (6.29 – 12.2)
OSWP-3	4.10 (2.71 – 6.79)	4.91 (2.77 – 8.02)
OSR-1	11.0 (8.62 – 13.2)	11.8 (5.85 – 14.7)
OSR-2	8.54 (4.95 – 11.3)	8.31 (3.19 – 13.8)
OSR-3	5.32 (3.63 – 6.97)	5.97 (4.46 – 8.71)
OSL-1	22.8 (15.5 – 34.3)	22.5 (15.4 – 31.1)
OSL-2	15.1 (9.84 – 23.6)	15.0 (10.9 – 21.4)
OSL-3	17.9 (12.7 – 24.6)	20.0 (14.6 – 29.5)
Pollen	20.3 (14.5 – 23.1)	22.1 (14.3 – 32.2)
Grain	3.15 (2.43 – 4.58)	3.34 (2.12 – 7.43)

1. Tissues were collected at the following growth stages (Ritchie, et al., 1997)
   a. OSL-1: V2 – V4; OSL-2: V8 – V10; OSL-3: R1
   b. OSR-1: V2 – V4; OSR-2: V5 – V6; OSR-3: R1
   c. OSWP-1: V10 – V12; OSWP-2: R4 – R5; OSWP-3: R6
   d. Pollen: during pollen shed (R1) e. Grain: R6/maturity
2. Protein levels are expressed on a dry weight basis (dwt)
3. ND = nor detected

Table 3. Cry3Bb1 and CP4EPSPS Protein Levels in MON 88017 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7

	Cry3Bb1		CP4 EPSPS	
	MON 88017	MON 89034× TC1507 x MON 88017× DAS-59122-7	MON 88017	MON 89034× TC1507 x MON 88017× DAS-59122-7
Tissue Type	Mean (Range) (µg/g dwt)	Mean (Range) (µg/g dwt)	Mean (Range) (µg/g dwt)	Mean (Range) (µg/g dwt)
OSWP-1	110 (70 – 190)	130 (73 – 220)	84 (47 – 130)	90 (74 – 120)
OSWP-2	40 (21 – 61)	48 (24 – 79)	37 (21 – 51)	42 (29 – 52)
OSWP-3	24 (6.9 – 58)	25 (8.9 – 49)	12 (3.1 – 26)	15 (5.1 – 27)
OSR-1	140 (81 – 190)	160 (95 – 260)	92 (58 – 120)	99 (71 – 110)
OSR-2	120 (71 – 170)	110 (63- 160)	77 (42 – 120)	72 (42 – 140)
OSR-3	50 (32 – 120)	65 (23 – 100)	34 (21 – 58)	35 (25 – 50)
OSL-1	330 (140 – 580)	310 (150 – 490)	170 (82 – 240)	190 (110 – 270)
OSL-2	110 (53 – 190)	130 (90 – 160)	120 (65 – 170)	140 (80 – 200)
OSL-3	140 (100 – 200)	170 (98 – 340)	180 (140 – 240)	160 (110 – 180)
Pollen	12 (8.1 – 16)	15 (7.5 – 24)	260 (170 – 340)	310 (190 – 480)
Grain	20 (12 – 38)	18 (10 – 16)	4.9 (3.3 - 7.4)	5.2 (3.5 - 7.1)

1. Tissues were collected at the following growth stages (Ritchie, et al., 1997)
   a. OSL-1: V2 – V4; OSL-2: V8 – V10; OSL-3: R1
   b. OSR-1: V2 – V4; OSR-2: V5 – V6; OSR-3: R1
   c. OSWP-1: V10 – V12; OSWP-2: R4 – R5; OSWP-3: R6
   d. Pollen: during pollen shed (R1) e. Grain: R6/maturity
2. Protein levels are expressed on a dry weight basis (dwt)

Table 4. Cry34Ab1 and Cry35Ab1 Protein Levels in DAS-59122-7 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7

	Cry34Ab1		Cry35Ab1	
	DAS-59122-7	MON 89034× TC1507 x MON 88017× DAS-59122-7	DAS-59122-7	MON 89034× TC1507 x MON 88017× DAS-59122-7
Tissue Type	Mean (Range) (µg/g dwt)[2]	Mean (Range) (µg/g dwt)	Mean (Range) (µg/g dwt)	Mean (Range) (µg/g dwt)
OSWP-1	106 (84.3 – 153)	98.4 (82.1 – 141)	58.3 (45.9 – 80.7)	55.8 (39.6 – 82.3)
OSWP-2	168 (117 – 233)	157 (113 – 194)	37.1 (26.8 – 54.4)	33.6 (27.7 – 42.3)
OSWP-3	127 (75.8 – 192)	134 (64.1 – 196)	17.8 (2.54 – 30.6)	18.9 (11.2 – 31.3)
OSR-1	105 (76.4 – 147)	105 (84.2 – 150)	52.9 (31.7 – 80.5)	49.5 (39.0 – 70.5)
OSR-2	90.9 (65.0 – 132)	90.1 (77.2 – 113)	41.5 (21.9 – 60.5)	39.3 (25.4 – 58.7)
OSR-3	85.4 (65.2 – 112)	84.6 (73.6 – 111)	18.3 (1.00 – 24.0)	18.9 (13.8 – 26.1)
OSL-1	92.3 (71.5 -118)	92.3 (71.9 – 141)	70.2 (48.2 – 86.0)	69.4 (45.4 – 96.1)
OSL-2	124 (101 – 176)	117 (91.1 – 159)	79.0 (60.0 – 113)	74.1 (51.4 – 101)
OSL-3	184 (143 – 252)	171 (135 – 279)	137 (105 -172)	121 (38.5 – 158)
Pollen	84.4 (68.0 – 97.8)	92.2 (77.5 – 117)	0.07 (ND -0.057)	0.09 (ND – 0.53)
Grain	66.5 (43.6 – 102)	62.6 (47.8 – 94.0)	1.86 (1.18 – 2.65)	1.69 (1.24 – 2.31)

1. Tissues were collected at the following growth stages (Ritchie, et al., 1997)
   a. OSL-1: V2 – V4; OSL-2: V8 – V10; OSL-3: R1
   b. OSR-1: V2 – V4; OSR-2: V5 – V6; OSR-3: R1
   c. OSWP-1: V10 – V12; OSWP-2: R4 – R5; OSWP-3: R6
   d. Pollen: during pollen shed (R1) e. Grain: R6/maturity
2. Protein levels are expressed on a dry weight basis (dwt)
3. ND = not detected

Table 5. PAT Protein Levels in TC1507, DAS-59122-7, and MON 89034 × TC1507 × MON 88017 × DAS-59122-7

	PAT		
	TC1507	DAS-59122-7	MON 89034× TC1507 x MON 88017× DAS-59122-7
Tissue Type	Mean (Range) (µg/g dwt)	Mean (Range) (µg/g dwt)	Mean (Range) (µg/g dwt)
OSWP-1	1.86 (1.01 – 2.76)	4.61 (2.90 – 6.72)	7.43 (4.33 – 11.9)
OSWP-2	0.52 (0.26 – 0.81)	1.58 (0.70 – 2.97)	2.51 (1.56 – 4.45)
OSWP-3	0.05 (ND – 0.17)	0.16 (ND – 0.49)	0.22 (ND – 0.80)
OSR-1	0.69 (0.20 – 0.90)	2.39 (0.77 – 3.53)	3.74 (2.39 – 7.43)
OSR-2	0.80 (0.40 – 1.17)	2.69 (1.02 – 4.77)	3.84 (1.80 – 6.05)
OSR-3	0.26 (0.16 – 0.44)	0.94 (0.28 – 1.62)	1.10 (0.36 – 1.85)
OSL-1	6.94 (4.70 – 9.94)	16.9 (11.4 – 22.9)	25.0 (18.1 – 34.9)
OSL-2	7.42 (4.81 – 12.4)	14.6 (9.43 – 19.6)	21.5 (14.6 – 26.4)
OSL-3	7.92 (5.15 – 12.3)	19.5 (13.2 – 24.3)	26.3 (17.0 – 32.4)
Pollen	ND (ND – ND)	ND (NO – ND)	ND (ND-ND)
Grain	ND (ND – ND)	0.049 (0.026 – 0.091)	0.050 (ND – 0.10)

1. Tissues were collected at the following growth stages (Ritchie, et al, 1997)
   a. OSL-1: V2 – V4; OSL-2: V8 – V10; OSL-3: R1
   b. OSR-1: V2 – V4; OSR-2: V5 - V6; OSR-1: R1
   c. OSWP-1: V10 – V12; OSWP-1: R4 – R5; OSWP-3: R6
   d. Pollen: during pollen shed (R1) e. Grain: R6/maturity
2. Protein levels are expressed on a dry weight basis (dwt)
3. ND = not detected

References

Ritchie, S.W., J.J. Hanway, and G.O. Benson. 1997. How a Corn Plant Develops: Special Report #48. Iowa State University of Science and Technology Cooperative Extension Service, Ames, Iowa

DATA EVALUATION RECORD

Primary Reviewer: Eric B. Lewis, M.S., Contract Reveiwer, Oak Ridge National Laboratories

EPA Secondary Reviewer: John L. Kough, Ph.D., Biologist JLK

STUDY TYPE:	Assessment of Protein Levels (Nonguideline)
MRID NO:	47444906
DP BARCODE:	DP355690
DECISION NO:	394799
SUBMISSION NO:	830991
TEST MATERIAL:	MON 89034 x TC1507 x MON 88017 x DAS-59122-7 (a.i., Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1)
STUDY NO:	MSL0021070; #06-01-52-18
SPONSOR:	Monsanto Company, 800 North Lindbergh Blvd., St. Louis, MO 63167
TESTING FACILITY:	Monsanto Company, 800 North Lindbergh Blvd., St. Louis, MO 63167
TITLE OF REPORT:	Assessment of Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS Protein Levels in the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7 Produced in U.S. Field Trials During 2006
AUTHORS:	Stillwell, L., and A. Silvanovich
STUDY COMPLETED:	November 30, 2007
CONFIDENTIALITY CLAIMS:	None.
GOOD LABORATORY PRACTICE:	A signed and dated compliance statement was provided. The study is in compliance with 40 CFR Part 160.

STUDY SUMMARY:	MON 89034 x TC1507 x MON 88017 x DAS-59122-7 is a combined trait corn that produces lepidopteran-active and coleopteran-active *Bacillus thuringiensis* (*Bt*) proteins, as well as the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) to confer tolerance to glyphosate herbicides. The levels of the lepidopteran-active Cry1A.105, Cry2Ab2, Cry3Bb1 proteins and the CP4 EPSPS protein were determined in tissues from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 plants grown at five US field sites in 2006. The test also included a conventional corn as a negative control and MON 89034 and MON 88017 corns as positive controls. Leaf, root, and whole plant samples were collected over the growing season, as well as pollen and grain samples at the appropriate times. The samples were extracted and analyzed using enzyme-linked immunosorbent assays (ELISA). The levels of the Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS proteins in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn were comparable to those in the appropriate MON 88017 or MON 89034 positive control.
CLASSIFICATION:	**Acceptable.**

Introduction

The combined trait corn MON 89034 x TC1507 x MON 88017 x DAS-59122-7 provides protection against lepidopteran and coleopteran insect pests. MON 89034 produces the lepidopteran–active *Bacillus thuringiensis* (*Bt*) Cry1A.105 and Cry2Ab2 proteins. TC1507 produces the lepidopteran-active *Bt* Cry1F protein, as well as the phosphinothricin acetyl transferase (PAT) protein from *Streptomyces viridochromogenes* to confer tolerance to glufosinate ammonium herbicides. MON 88017 produce the coleopteran-active *Bt* Cry3Bb1 protein and the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) to confer tolerance to glyphosate herbicides. DAS-59122-7 contains the coleopteran-active Cry34Ab1 and Cry35Ab1 proteins, as well as the PAT protein.

Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS protein levels were determined in tissues from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 plants, as well as control and reference corn plants, grown at five US field sites in 2006 under Monsanto production plan 06-01-52-04. The sites represented geographical regions where corn is grown commercially.

Test Materials

The test substance was MON 89034 x TC1507 x MON 88017 x DAS-59122-7 (starting seed lot GLP-0604-17108-S).

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The negative control substance was a conventional corn with a similar genetic background the test substance plants (starting seed lot GLP-0604-17109-S).

The positive control substances were MON 88017 (starting seed lot GLP-06-4-17100-S) and MON 89034 (starting seed lot GLP-0604-17104-S).

The identities of the test and control substances were confirmed by verifying the chain of custody documentation prior to analysis. Also, event-specific polymerase chain reaction (PCR) analyses were conducted on seed and grain samples.

The reference standards were four *E. coli*-produced protein standards:

Cry1A.105 (Lot No. 20-100086), with a purity-corrected concentration of 1.0 mg/mL by amino acid composition analysis. The purity was 80% as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and densitometric analysis.

Cry2Ab2 (Lot No. 20-100071), with a purity-corrected concentration of 0.4 mg/mL by amino acid composition analysis. The purity was 87% as determined by SDS-PAGE and densitometric analysis.

Cry3Bb1 (Lot No. 20-100084), with a purity-corrected concentration of 1.0 mg/mL by amino acid composition analysis. The purity was 86% as determined by SDS-PAGE and densitometric analysis.

CP4 EPSPS (Lot No. 20-100015), with a purity-corrected concentration of 3.7 mg/mL by amino acid composition analysis. The purity was 97% as determined by SDS-PAGE and densitometric analysis.

Summary of Methods

The field sites were located in Jefferson County, Iowa, Greene County, Iowa, Stark County, Illinois, Clinton County, Illinois, and York County, Nebraska. At each site, three replicates of each corn variety were planted in a randomized complete block design. Over-season leaf (OSL 1-3), over-season root (OSR 1-3), over season whole plant (OSWP 1-3), pollen and grain tissues were collected from each replicate at all sites. The over season samples were collected at three different growth stages (Ritchie, et al., 1997): for leaves, OSL-1 at stages V2-V4, OSL-2 at V8-V10, and OSL-3 at R1; for roots, OSR-1 at stages V2-V4, OSR-2 at V5-V6, and OSR-3 at R1; and for whole plants, OSWP-1 at stages V10-V12, OSWP-2 at R4-R5, and OSWP-3 at R6. All field samples except grain were stored and shipped on dry ice to the Monsanto processing facility. Grain samples were stored and shipped at ambient temperature. Processed tissues were stored at -80°C until they were shipped on dry ice to Monsanto's analytical facility, where they were stored at -80°C.

The Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS proteins were extracted from corn tissues according to Monsanto SOPs BR-ME-1-1027-1, BR-ME-1026-1, BR-ME-0884-03, and BR-ME-0197-06, respectively. Extraction procedures for each protein and tissue type and validation of the assays are provided in Appendices 2-5 of MRID 47444906. The tissues were kept on dry ice and extracted with a Harbil mixer. Insolubles were removed using a Serum Filter System (Fisher Scientific) or by centrifugation. The extracts were aliquoted and stored at -80°C until analysis.

Analysis was by enzyme linked immunosorbent assays (ELISA). The ELISA reagents and methods are described on pp. 18-21 of MRID 47444906. Moisture content was measured for all tissue types using an IR-200 Moisture Analyzer (Denver Instrument Co., Arvada, CO), and the protein levels were converted and reported on a dry weight basis.

A microplate spectrometer (SPECTRAmax Plus, Molecular Devices, Sunnyvale, CA) was used for analysis of the Cry1A.105 and Cry2Ab2 ELISA plates. Cry3Bb1 and CP4 EPSPS plates were analyzed on a SPECTRAmax Plus or a SPECTRAFluor Plus (Tecan, Research Triangle Park, NC) microplate spectrometer. All concentrations were determined by optical absorbance at 450 nm with a simultaneous reference reading at 620-650 nm. Data reduction analyses were performed using Molecular Devices SOFTmax PRO v. 4.7.1. Absorbance readings and protein concentrations were fitted with a four-parameter logistic curve fit. Microsoft Excel 2002 (Microsoft, Redmond, WA) was used to calculate the dry weight-corrected protein levels in the corn tissues.

Summary of Results

The Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS levels in tissues of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 and the appropriate MON 89034 or MON 88017 positive control are summarized in Tables 1 thru 16.

The mean Cry1A.105 level (dry wt) in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 across all sites was 4.3 µg/g in grain, 19 µg/g in forage (OSWP-2), 14 µg/g in pollen, 140 µg/g in OSL-1, and 73 µg/g in OSR-1.

The mean Cry2Ab2 level (dry wt) in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 across all sites was 5.7 µg/g in grain, 29 µg/g in forage (OSWP-2), 0.81 µg/g in pollen, 220 µg/g in OSL-1, and 54 µg/g in OSR-1.

The mean Cry3Bb1 level (dry wt) in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 across all sites was 18 µg/g in grain, 48 µg/g in forage (OSWP-2), 15 µg/g in pollen, 310 µg/g in OSL-1, and 160 µg/g in OSR-1.

The mean CP4 EPSPS level (dry wt) in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 across all sites was 5.2 µg/g in grain, 42 µg/g in forage (OSWP-2), 310 µg/g in pollen, 190 µg/g in OSL-1, and 99 µg/g in OSR-1.

The levels of Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS in all tissues from the conventional control corn were below the assay LOQ or LOD for each tissue type.

Study Authors' Conclusions

The study authors concluded that the levels of Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS proteins found in tissues of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 were comparable to those protein levels in tissues of the appropriate MON 89034 or MON 88017 positive control.

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Table 1. Summary of Cry1A.105 Protein Levels in Corn Leaf Tissues Collected from MON 89034 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 89034		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
OSL-1	21 (5.6) 14 - 30	130 (41) 85 - 200	22 (4.6) 16 - 32	140 (33) 100 - 210
OSL-2	13 (3.4) 8.5 - 19[6]	63 (13) 44 - 89	16 (4.8) 9.9 - 25	79 (22) 47 - 120
OSL-3	20 (7.2) 11 - 33	77 (28) 39 - 140	21 (6.7) 14 - 34	80 (24) 53 - 120

1. Tissues were collected at the following growth stages (Ritchie et al., 1997):
   a. OSL-1: V2-V4
   b. OSL-2: V8-V10
   c. OSL-3: R1
2. The mean and standard deviation were calculated across sites (MON 89034, n=14 except OSL-2, n=13; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.
6. One OSL-2 sample at site 1A-2 was <LOD. This sample is not included in the average or range calculations.

Table 2. Summary of Cry1A.105 Protein Levels in Corn Root Tissues Collected from MON 89034 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 89034		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
OSR-1	8.1 (2.7) 5.7 - 26[6]	70 (25) 48 - 240	8.4 (1.5) 6.0 - 12	73 (12) 55 - 100
OSR-2	6.0 (1.3) 4.1 - 8.9[7]	47 (13) 31 - 81	6.1 (1.1) 4.4 - 8.7[8]	47 (9.7) 32 - 64
OSR-3	3.9 (0.76) 2.8 - 5.5	20 (5.6) 15 - 34	4.0 (1.3) 2.2 - 6.5	21 (6.2) 11 - 30

1. Tissues were collected at the following growth stages (Ritchie et al., 1997):
   a. OSR-1: V2-V4
   b. OSR-2 V5-V6
   c. OSR-3 R1
2. The mean and standard deviation were calculated across sites (MON 89034 OSR-1 and OSR-2, n=13; MON 89034 OSR-3, n=14; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15 except OSR-2, n=14).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.
6. One OSR-1 sample at site IL-1 was <LOD. This sample is not included in the average or range calculations.
7. One OSR-2 sample at site IA-2 was <LOD. This sample is not included in the average or range calculations.
8. One OSR-2 sample at site NE was <LOD. This sample is not included in the average or range calculations.

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Table 3. Summary of Cry1A.105 Protein Levels in Corn Whole Plant Tissues Collected from MON 89034 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 89034		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
OSWP-1	4.9 (1.9) 1.9 - 7.9	43 (14) 21 - 66	6.2 (2.4) 2.8 - 10	53 (17) 32 - 86
OSWP-2 (forage)	5.2 (2.1) 2.0 - 8.7	18 (7.5) 6.9 - 31	5.7 (1.8) 2.3 - 8.7	19 (5.2) 8.1 - 26
OSWP-3	4.3 (2.3) 1.9 - 9.6	8.8 (5.5) 3.9 - 22	5.4 (2.5) 2.1 - 9.8	11 (5.6) 3.8 - 21

1. Tissues were collected at the following growth stages (Ritchie et al., 1997):
   a. OSWP-1: V10-V12
   b. OSWP-2 (forage): R4-R5
   c. OSWP-3: R6
2. The mean and standard deviation were calculated across sites (MON 89034, n=14; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.

Table 4. Summary of Cry1A.105 Protein Levels in Corn Pollen and Grain Collected from MON 89034 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 89034		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
Pollen	4.7 (1.8) 2.6 - 8.6	7.1 (2.0) 5.1 - 12	9.1 (2.8) 5.1 - 16	14 (3.0) 7.8 - 21
Grain	2.5 (0.35) 1.6 - 3.1	2.8 (0.40) 1.7 - 3.5	3.8 (0.39) 3.0 - 4.3	4.3 (0.44) 3.4 - 4.9

1. Tissues were collected at the following growth stages:
   a. Pollen: during pollen shed (R1)
   b. Grain: R6/maturity
2. The mean and standard deviation were calculated across sites ((MON 89034, n=14; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing tbe fwt. by tbe dry weight conversion factors obtained from moisture analysis data.

Table 5. Summary of Cry2Ab2 Protein Levels in Corn Leaf Tissues Collected from MON 89034 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 89034		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
OSL-1	33 (7.7) 23 - 45	210 (53) 140 - 290	34 (10) 23 - 53	220 (69) 140 - 350
OSL-2	27 (14) 12 - 69[6]	140 (64) 76 - 330	28 (8.7) 9.7 - 41	140 (44) 60 - 200
OSL-3	48 (7.6) 34 - 60	180 (28) 120 - 240	48 (7.8) 36 - 60	180 (26) 140 - 220

1. Tissues were collected at the following growth stages (Ritchie et al., 1997):
   a. OSL-1: V2-V4
   b. OSL-2: V8-V10
   c. OSL-3: R1
2. The mean and standard deviation were calculated across sites (MON 89034 OSL-2, n=13; MON 89034 OSL-1 and OSL-3, n=14; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.
6. One OSL-2 sample at site IA-2 was <LOD. This sample is not included in the average or range calculations.

Table 6. Summary of Cry2Ab2 Protein Levels in Corn Root Tissues Collected from MON 89034 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 89034		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
OSR-1	5.7 (1.6) 3.3 - 8.8[6]	50 (15) 28 - 80	6.2 (1.7) 3.7 - 8.7	54 (14) 32 - 76
OSR-2	8.6 (4.1) 2.9 - 14[7]	69 (35) 22 - 120	7.7 (3.9) 3.4 – 18[8]	58 (25) 26 - 120
OSR-3	3.1 (2.6) 0.85 - 8.9	16 (12) 4.0 - 40	3.5 (3.0) 0.85 - 11	18 (14) 4.1 - 49

1. Tissues were collected at the following growth stages (Ritchie et al., 1997):
   a. OSR-1: V2-V4
   b. OSR-2 V5-V6
   c. OSR-3 R1
2. The mean and standard deviation were calculated across sites (MON 89034 OSR-1 and OSR-2, n=13; MON 89034 OSR-3, n=14; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15 except OSR-2, n=14).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.
6. One OSR-1 sample at site IL-1 was <LOQ. This sample is not included in the average or range calculations.
7. One OSR-2 sample at site IA-2 was <LOQ. This sample is not included in the average or range calculations.
8. One OSR-2 sample at site NE was <LOQ. This sample is not included in the average or range calculations.

Table 7. Summary of Cry2Ab2 Protein Levels in Corn Whole Plant Tissues Collected from MON 89034 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 89034		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
OSWP-1	6.1 (3.3) 1.9 - 14	54 (30) 21 - 130	6.1 (2.5) 1.9 - 9.6	53 (20) 21 - 80
OSWP-2 (forage)	8.6 (3.4) 3.5 - 15	29 (12) 12 - 54	9.1 (4.3) 3.2 - 19	29 (11) 11 - 53
OSWP-3	7.3 (4.1) 1.8 - 16	15 (9.6) 3.6 - 38	7.9 (5.3) 2.4 - 18	16 (12) 4.5 - 41

1. Tissues were collected at the following growth stages (Ritchie et al.; 1997):
   a. OSWP-1: V10-V12
   b. OSWP-2 (forage): R4-R5
   c. OSWP-3: R6
2. The mean and standard deviation were calculated across sites (MON 89034 n=14; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.

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Table 8. Summary of Cry2Ab2 Protein Levels in Corn Pollen and Grain Collected from MON 89034 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 89034		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
Pollen	0.53 (0.42) 0.16 - 1.8	0.76 (0.46) 0.31 - 2.2	0.56 (0.47) 0.12 - 1.8	0.81 (0.54) 0.18 - 2.3
Grain	4.9 (1.0) 2.4 - 6.3	5.6 (1.1) 2.7 - 7.1	5.0 (0.84) 3.6 - 6.7	5.7 (0.94) 4.1 - 7.5

1. Tissues were collected at the following growth stages:
   a. Pollen: during pollen shed (R1)
   b. Grain: R6/maturity
2. The mean and standard deviation were calculated across sites (MON 89034 n=14; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15 except Pollen, n=13).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.

Table 9. Summary of Cry3Bb1 Protein Levels in Corn Leaf Tissues Collected from MON 88017 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 88017		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
OSL-1	51 (22) 23 - 87	330 (150) 140 - 580	48 (19) 25 - 73	310 (130) 150 - 490
OSL-2	23 (7.4) 8.5 - 40	110 (34) 53 - 190	25 (3.1) 18 - 29	130 (19) 90 - 160
OSL-3	38 (5.7) 29 - 47	140 (25) 100 - 200	45 (20) 23 - 92	170 (69) 98 - 340

1. Tissues were collected at the following growth stages (Ritchie et al., 1997):
   a. OSL-1: V2-V4
   b. OSL-2: V8-V10
   c. OSL-3: R1
2. The mean and standard deviation were calculated across sites (MON 88017 n=14; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.

Table 10. Summary of Cry3Bb1 Protein Levels in Corn Root Tissues Collected from MON 88017a nd MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 88017		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
OSR-1	16 (3.3) 9.8 - 21	140 (32) 81 - 190	19 (6.0) 11 - 31	160 (53) 95 - 260
OSR-2	16 (4.5) 9.9 - 26	120 (35) 71 - 170	14 (4.8) 8.8 - 23[6]	110 (35) 63 - 160
OSR-3	9.7 (3.2) 6.4 - 19	50 (22) 32 - 120	13 (5.1) 4.3 - 22	65 (25) 23 - 100

1. Tissues were collected at the following growth stages (Ritchie et al., 1997):
   a. OSR-1: V2-V4
   b. OSR-2 V5-V6
   c. OSR-3 R1
2. The mean and standard deviation were calculated across sites (MON 88017 n=14; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15 except OSR-2, n=14).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.
6. One sample at site NE was >100-fold below the mean µg/g fwt. for OSR-2. This sample is not included in the average or range calculations.

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Table 11. Summary of Cry3Bb1 Protein Levels in Corn Whole Plant Tissues Collected from MON 88017 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 88017		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
OSWP-1	12 (4.8) 6.3 - 23	110 (38) 70 - 190	15 (5.5) 8.0 - 26	130 (40) 73 - 220
OSWP-2 (forage)	12 (4.5) 5.8 - 19	40 (14) 21 - 61	14 (5.1) 6.6 - 25	48 (16) 24 - 79
OSWP-3	12 (7.2) 3.4 - 25	24 (16) 6.9 - 58	13 (5.8) 5.0 - 25	25 (12) 8.9 - 49

1. Tissues were collected at the following growth stages (Ritchie et al., 1997):
   a. OSWP-1: V10-V12
   b. OSWP-2 (forage): R4-R5
   c. OSWP-3: R6
2. The mean and standard deviation were calculated across sites (MON 88017 n=14; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.

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Table 12. Summary of Cry3Bb1 Protein Levels in Corn Pollen and Grain Collected from MON 88017 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 88017		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
Pollen	8.2 (2.6) 4.3 - 13	12 (2.5) 8.1 - 16	9.6 (3.9) 4.9 - 19	15 (4.5) 7.5 - 24
Grain	17 (6.2) 10 - 34	20 (6.9) 12 - 38	16 (4.0) 9.1 - 23	18 (4.6) 10 - 26

1. Tissues were collected at the following growth stages:
   a. Pollen: during pollen shed (R1)
   b. Grain: R6/maturity
2. The mean and standard deviation were calculated across sites (MON 88017 n=14; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.

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Table 13. Summary of CP4 EPSPS Protein Levels in Corn Leaf Tissues Collected from MON 88017 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 88017		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
OSL-1	27 (6.1) 13 - 38	170 (43) 82 - 240	29 (6.9) 19 - 41	190 (49) 110 - 270
OSL-2	23 (6.6) 14 - 35	120 (28) 65 - 170	28 (7.4) 16 - 42	140 (36) 80 - 200
OSL-3	49 (12) 33 - 69	180 (32) 140 - 240	42 (8.3) 25 - 54	160 (21) 110 - 180

1. Tissues were collected at the following growth stages (Ritchie et al., 1997):
   a. OSL-1: V2-V4
   b. OSL-2: V8-V10
   c. OSL-3: R1
2. The mean and standard deviation were calculated across sites (MON 88017 n=14; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.

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Table 14. Summary of CP4 EPSPS Protein Levels in Corn Root Tissues Collected from MON 88017 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 88017		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
OSR-1	11 (2.4) 6.9 - 15	92 (20) 58 - 120	12 (1.8) 8.2 - 14	99 (14) 71 - 110
OSR-2	9.9 (3.3) 5.4 - 19	77 (23) 42 - 120	9.4 (3.9) 5.8 - 21[6]	72 (27) 42 - 140
OSR-3	6.7 (1.5) 3.9 - 9.5	34 (8.7) 21 - 58	6.9 (1.7) 4.7 - 11	35 (7.6) 25 - 50

1. Tissues were collected at the following growth stages (Ritchie et al., 1997):
   a. OSR-1: V2-V4
   b. OSR-2 V5-V6
   c. OSR-3 R1
2. The mean and standard deviation were calculated across sites (n=15, except MON 89034 × TC1507 × MON 88017 × DAS-59122-7 OSR-2, n=14).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.
6. One OSR-2 sample at site NE was <LOD. This sample is not included in the average or range calculations.

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Table 15. Summary of CP4 EPSPS Protein Levels in Corn Whole Plant Tissues Collected from MON 88017 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 88017		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
OSWP-1	9.6 (3.3) 5.4 - 16	84 (26) 47 - 130	10 (2.4) 6.7 - 14	90 (15) 74 - 120
OSWP-2 (forage)	11 (2.4) 7.5 - 15	37 (8.6) 21 - 51	13 (2.8) 8.9 - 18	42 (7.7) 29 - 52
OSWP-3	6.1 (3.4) 1.9 - 13	12 (7.7) 3.1 - 26	7.3 (3.0) 2.5 - 12	15 (7.3) 5.1 - 27

1. Tissues were collected at the following growth stages (Ritchie et al., 1997):
   a. OSWP-1: V10-V12
   b. OSWP-2 (forage): R4-R5
   c. OSWP-3: R6
2. The mean and standard deviation were calculated across sites (MON 88017 n=14; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.

Table 16. Summary of CP4 EPSPS Protein Levels in Corn Pollen and Grain Collected from MON 88017 and MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials Conducted in 2006

Tissue Type[1]	MON 88017		MON 89034 × TC1507 × MON 88017 × DAS-59122-7	
	Mean (SD)[2] Range[3] (µg/g fwt.)[4]	Mean (SD) Range (µg/g dwt.)[5]	Mean (SD) Range (µg/g fwt.)	Mean (SD) Range (µg/g dwt.)
Pollen	170 (25) 110 - 210	260 (40) 170 - 340	200 (45) 130 – 260	310 (86) 190 – 480
Grain	4.3 (1.0) 3.0 – 6.6	4.9 (1.2) 3.3 – 7.4	4.6 (0.98) 3.1 – 6.4	5.2 (1.1) 3.5 – 7.1

1. Tissues were collected at the following growth stages:
   a. Pollen: during pollen shed (R1)
   b. Grain: R6/maturity
2. The mean and standard deviation were calculated across sites (MON 88017 Grain, n=14; MON 88017 Pollen, n=13; MON 89034 × TC1507 × MON 88017 × DAS-59122-7 n=15).
3. Minimum and maximum values were determined for each tissue type across sites.
4. Protein levels are expressed as microgram (µg) of protein per gram (g) of tissue on a fresh weight (fwt.) basis.
5. Protein levels are expressed as µg/g on a dry weight (dwt.) basis. The dry weight values were calculated by dividing the fwt. by the dry weight conversion factors obtained from moisture analysis data.

References

Ritchie, S.W., J.J. Hanway, and G.O. Benson. 1997. How a Corn Plant Develops: Special Report #48. Iowa State University of Science and Technology Cooperative Extension Service, Ames, Iowa

DATA EVALUATION RECORD

Primary Reviewer: Eric B. Lewis, M.S., Contract Reviewer, Oak Ridge National Laboratories
EPA Secondary Reviewer: John L. Kough, Ph.D., Biologist

STUDY TYPE:	Assessment of Protein Levels (Nonguideline)
MRID NO:	47444907
DP BARCODE:	DP355690
DECISION NO:	394799
SUBMISSION NO:	830991
TEST MATERIAL:	MON 89034 x TC1507 x MON 88017 x DAS-59122-7 (a.i., Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1)
STUDY NO:	061026
SPONSOR:	Monsanto Company, 800 North Lindbergh Blvd., St. Louis, MO 63167
TESTING FACILITY:	Regulatory Laboratories – Indianapolis Lab, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268-1054
TITLE OF REPORT:	Cry34Ab1, Cry35Ab1, Cry1F and PAP Protein Levels in Hybrid Maize TC1507, DAS-59122-7, MON 89034 x TC1507 x MON 88017 x DAS-59122-7, and a Conventional Corn Control from the Monsanto 2006 Production Plan 06-01-52-04
AUTHOR:	Phillips, A.M.
STUDY COMPLETED:	January 18, 2008 (report date)
CONFIDENTIALITY CLAIMS:	None.
GOOD LABORATORY PRACTICE:	A signed and dated compliance statement was provided. The study was conducted in compliance with 40 CFR Part 160, with the exception that the original containers of samples shipped from Monsanto were not retained as test substance containers.
STUDY SUMMARY:	MON 89034 x TC1507 x MON 88017 x DAS-59122-7 is a combined trait corn that produces lepidopteran-active and coleopteran-active *Bacillus thuringiensis* (*Bt*) proteins, as well as the phosphinothricin acetyl transferase (PAT) protein from *Streptomyces viridochromogenes* to confer tolerance to glufosinate ammonium herbicides. The levels of the coleopteran-active *Bacillus thuringiensis* (*Bt*) proteins Cry34Ab1, Cry35Ab1, and Cry1F, and the PAT protein were determined in tissues from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 plants grown at five US field sites in 2006. The test also included a conventional corn as a negative control and TC1507 and DAS-5912207 corns as positive controls. Leaf, root, and whole plant samples were collected over the growing

	season, as well as pollen and grain samples at the appropriate times. The samples were extracted and analyzed using enzyme-linked immunosorbent assays. The results indicate that the levels of Cry34Ab1, Cry35Ab1, and Cry1F in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 were comparable to the levels produced in the appropriate TC1507 or DAS-59122-7 control corn. The level of PAT in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 was higher in the combined trait products compared to TC1507 and DAS-59122-7, likely due to the presence of multiple copies of the *pat* gene in the stacks (one from each of the DAS parent lines).
CLASSIFICATION:	**Acceptable.**

Introduction

The combined trait corn MON 89034 x TC1507 x MON 88017 x DAS-59122-7 provides protection against lepidopteran and coleopteran insect tests. MON 89034 produces the lepidopteran–active *Bacillus thuringiensis* (*Bt*) Cry1A.105 and Cry2Ab2 proteins. TC1507 produces the lepidopteran-active *Bt* Cry1F protein, as well as the phosphinothricin acetyl transferase (PAT) protein from *Streptomyces viridochromogenes* to confer tolerance to glufosinate ammonium herbicides. MON 88017 produce the coleopteran-active *Bt* Cry3Bb1 protein and the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) to confer tolerance to glyphosate herbicides. DAS-59122-7 contains the coleopteran-active Cry34Ab1 and Cry35Ab1 proteins, as well as the PAT protein.

Cry34Ab1, Cry35Ab1, Cry1F, and PAT protein levels were determined in tissues from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 plants, as well as control and reference corn plants, grown at five US field sites in 2006. The sites represented geographical regions where corn is grown commercially.

Test Materials

The test substance was MON 89034 x TC1507 x MON 88017 x DAS-59122-7.

The negative control substance was a conventional corn (not identified in MRID 47444907).

The positive control substances were DAS-59122-7 and TC1507.

The reference standards were:

Cry34Ab1 protein standard (Test Substance No. 104874), at a concentration of 0.248 mg/mL

Cry35Ab1 protein standard (Test Substance No. 104066), at a concentration of 0.187 mg/mL

Cry1F protein standard (Test Substance No. 104301), at a concentration of 0.164 mg/mL, and

PAT protein standard (Test Substance No. 105742), at a concentration of 300 µg/mL.

Certificates of analysis for the reference standards are cited on p. 20 of MRID 47444907.

Summary of Methods

The field sites were located in Jefferson County, Iowa, Greene County, Iowa, Stark County, Illinois, Clinton County, Illinois, and York County, Nebraska. At each site, three replicates of each corn were planted in a randomized complete block design. Over-season leaf (OSL 1-3), over-season root (OSR 1-3), over season whole plant (OSWP 1-3), pollen and grain tissues were collected at all sites. The over season samples were collected at three different growth stages (Ritchie, et al., 1997): for leaves, OSL-1 at stages V2-V4, OSL-2 at V8-V10, and OSL-3 at R1; for roots, OSR-1 at stages V2-V4, OSR-2 at V5-V6, and OSR-3 at R1; and for whole plants, OSWP-1 at stages V10-V12, OSWP-2 at R4-R5, and OSWP-3 at R6. Pollen samples were collected during pollen shed, and grain samples were collected at physiological maturity. The samples were collected in association with Monsanto production plan 06-01-52-04 and sent to Dow AgroSciences Regulatory Laboratory where they were analyzed for the Cry34Ab1, Cry35Ab1, Cry1F, and PAT proteins by enzyme linked immunosorbent assay (ELISA). All tissue samples except pollen were processed at Monsanto prior to shipment to Dow AgroSciences. The pollen samples were shipped directly to Dow AgroSciences from the field sites. Upon receipt at Dow AgroSciences, all samples except pollen were prepared by lyophilizing and grinding to a fine powder using a Geno Grinder. Pollen samples were lyophilized with no further preparation. After preparation, the samples were stored at -80°C.

The tissue samples were analyzed for Cry34Ab1, Cry35Ab1, Cry1F, and PAT according to DowAgrosciences methods GRM 04.07, GRM 03.13, GRM 02.30, and the method referenced in DAS databook C2148, respectively. The methods are described on pp. 13-16 of MRID 47444907. Absorbance at 450 nm minus 650 nm was measured using a Molecular Devices V-max or SpectraMax 190 plate reader. A calibration curve was generated using the appropriate standard and the applicable protein concentration in the unknown samples was calculated from the polynomial regression equation using Soft-MAX Pro software. Samples were analyzed in duplicate wells and the average concentration was reported.

Summary of Results

The Cry34Ab1, Cry35Ab1, Cry1F, and PAT protein concentrations (averaged across sites) in the corn matrices are summarized in Tables 1-4.

In TC1507, the Cry1F concentration ranged from 2.4 ng/mg in grain to 34 ng/mg in OSL-1. The PAT concentration ranged from ND in pollen, grain, and OSWP-3 to 12 ng/mg in OSL-2.

In DAS-59122-7, the Cry34Ab1 concentration ranged from 44 ng/mg in grain to 250 ng/mg in OSL-3. Cry35Ab1 concentrations ranged from ND in pollen to 170 ng/mg in OSL-3. PAT concentrations ranged from ND in pollen and OSWP-3 to 24 ng/mg in OSL-3.

For MON 89034 x TC1507 x MON 88017 x DAS-59122-7, Cry34Ab1 concentrations ranged from 48 ng/mg in grain to 280 ng/mg in OSL-3. Cry35Ab1 ranged from ND in pollen to 160 ng/mg in

OSL-3. Cry1F concentrations ranged from 2.1 ng/mg in grain to 32 ng/mg in pollen. PAT concentrations ranged from ND in pollen, grain, and OSWP-3 to 35 ng/mg in OSL-1.

Cry34Ab1, Cry35Ab1, and Cry1F expression levels were similar in the combined trait products compared to the TC1507 and DAS-59122-7. PAT expression was higher in the combined trait products compared to TC1507 and DAS-59122-7. This was likely due to the presence of multiple copies of the *pat* gene in the stacks (one from each of the DAS parent lines).

Cry35Ab1, Cry1F, and PAT were not detected above the method LOQ in any control samples, with the exception of on OSWP-2 sample. Three pollen control samples, two OSL-3 control samples, and one OSWP-3 control sample contained Cry34Ab1 above the method LOQ.

TABLE 1. Summary of Cry34Ab1 levels in corn tissues

Maize tissue[1]	Maize line	Cry34Ab1 (ng/mg dwt)[2]		
		Mean	Std dev	Range[3]
OSL-1	DAS-59122-7	92.3	16.7	71.5 – 118
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	92.3	19.5	71.9 - 141
OSL-2	DAS-59122-7	124	23.8	101 – 176[3]
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	117	21.3	91.1 – 159
OSL-3	DAS-59122-7	184	28.3	143 – 252
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	171	42.7	115 – 279
OSR-1	DAS-59122-7	105	18.6	76.4 – 147[3]
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	105	21.0	84.2 – 150[3]
OSR-2	DAS-59122-7	90.9	17.3	65.0 – 132
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	90.1	8.82	77.2 – 113[3]
OSR-3	DAS-59122-7	85.4	12.0	65.2 – 112
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	84.6	10.9	73.6 – 111
OSWP-1	DAS-59122-7	106	20.2	84.3 – 153
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	98.4	16.6	82.1 – 141
OSWP-2	DAS-59122-7	168	32.4	117 – 233
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	157	22.3	113 – 194
OSWP-3	DAS-59122-7	127	38.7	75.8 – 192
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	134	44.9	64.1 – 196
Pollen	DAS-59122-7	84.4	7.21	68.0 – 97.8
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	92.2	11.3	77.5 – 117
Grain	DAS-59122-7	66.5	14.1	43.6 – 102
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	62.6	11.4	47.8 – 94.0

Data from pp. 25-26, MRID 47444907

[1]Tissues collected at the following approximate growth stages (Ritchie et al., 1997):
OSL-1: V2-V4; OSL-2: V8-V10; OSL-3: R1
OSR-1: V2-V4; OSR-2: V5-V6; OSR-3: R1
OSWP-1: V10-V12; OSWP-2: R4-R5; OSWP-3: R6
Pollen: During pollen shed (R1)
Grain: R6/maturity

[2]Protein levels are expressed as ng/mg dry weight

[3]Minimum and maximum values were determined for each tissue across sites (n=15)
For OSL-2, DAS-59122-7 range does not include 1 sample at ND
For OSR-1, DAS-59122-7 n=14 and MON 89034 x TC1507 x MON 88017 x DAS-59122-7 n=13
For OSR-2, MON 89034 x TC1507 x MON 88017 x DAS-59122-7 range does not include one low sample at 0.12 ng/mg.

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TABLE 2. Summary of Cry35Ab1 levels in corn tissues				
Maize tissue[1]	Maize line	Cry35Ab1 (ng/mg dwt)[2]		
		Mean	Std dev	Range[3]
OSL-1	DAS-59122-7	70.2	13.1	48.2 – 86.0
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	69.4	17.7	45.4 – 96.1
OSL-2	DAS-59122-7	79.0	16.9	60.0 – 113[3]
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	74.1	14.0	51.4 – 101
OSL-3	DAS-59122-7	137	20.0	105 – 172
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	121	31.7	38.5 – 158
OSR-1	DAS-59122-7	52.9	15.0	31.7 - 80.5[3]
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	49.5	11.1	39.0 – 70.5[3]
OSR-2	DAS-59122-7	41.5	13.2	21.9 – 60.5
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	39.3	8.81	25.4 - 58.7[3]
OSR-3	DAS-59122-7	18.3	5.40	1.00 – 24.0
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	18.9	3.93	13.8 – 26.1
OSWP-1	DAS-59122-7	58.3	9.18	45.9 – 80.7
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	55.8	11.9	39.6 – 82.3
OSWP-2	DAS-59122-7	37.1	6.78	26.8 – 54.4
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	33.6	4.67	27.7 – 42.3
OSWP-3	DAS-59122-7	17.8	8.61	2.54 – 30.6
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	18.9	6.84	11.2 – 31.3
Pollen	DAS-59122-7	(0.03)	0.01	ND – 0.057
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	0.09	0.14	ND – 0.53
Grain	DAS-59122-7	1.86	0.37	1.18 – 2.65
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	1.69	0.28	1.24 – 2.31

Data from pp. 27-28, MRID 47444907

[1]Tissues collected at the following approximate growth stages (Ritchie et al., 1997):
OSL-1: V2-V4; OSL-2: V8-V10; OSL-3: R1
OSR-1: V2-V4; OSR-2: V5-V6; OSR-3: R1
OSWP-1: V10-V12; OSWP-2: R4-R5; OSWP-3: R6
Pollen: During pollen shed (R1)
Grain: R6/maturity

[2]Protein levels are expressed as ng/mg dry weight

[3]Minimum and maximum values were determined for each tissue across sites (n=15)
For OSL-2, DAS-59122-7 range does not include 1 sample at ND
For OSR-1, DAS-59122-7 n=14 and MON 89034 x TC1507 x MON 88017 x DAS-59122-7 n=13
For OSR-2, MON 89034 x TC1507 x MON 88017 x DAS-59122-7 range does not include one low sample at 0.05 ng/mg.
Values in parentheses are between the method LOD and LOQ.

TABLE 3. Summary of Cry1F levels in corn tissues				
Maize tissue[1]	**Maize line**	Cry1F (ng/mg dwt)[2]		
		Mean	**Std dev**	**Range[3]**
OSL-1	TC-1507	22.8	5.83	15.5 – 34.3
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	22.5	5.57	15.4 – 31.1
OSL-2	TC-1507	15.1	4.37	9.84 – 23.6
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	15.0	3.40	10.9 – 21.4
OSL-3	TC-1507	17.9	3.52	12.7 – 24.6
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	20.0	4.34	14.6 – 29.5
OSR-1	TC-1507	11.0	1.51	8.62 – 13.2[3]
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	11.8	2.37	5.85 – 14.7[3]
OSR-2	TC-1507	8.54	1.97	4.95 – 11.3
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	8.31	2.92	3.19 – 13.8[3]
OSR-3	TC-1507	5.32	1.06	3.63 – 6.97
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	5.97	1.26	4.46 – 8.71
OSWP-1	TC-1507	10.0	1.97	6.85 – 13.0
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	11.2	2.27	7.53 – 15.8
OSWP-2	TC-1507	7.69	1.85	4.65 – 10.6
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	9.00	1.76	6.29 – 12.2
OSWP-3	TC-1507	4.10	1.29	2.71 – 6.79
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	4.91	1.33	2.77 – 8.02
Pollen	TC-1507	20.3	2.99	14.5 – 23.1
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	22.1	4.51	14.3 – 32.2
Grain	TC-1507	3.15	0.63	2.43 – 4.58
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	3.34	1.23	2.12 – 7.43

Data from pp. 29-30, MRID 47444907

[1]Tissues collected at the following approximate growth stages (Ritchie et al., 1997):
OSL-1: V2-V4; OSL-2: V8-V10; OSL-3: R1
OSR-1: V2-V4; OSR-2: V5-V6; OSR-3: R1
OSWP-1: V10-V12; OSWP-2: R4-R5; OSWP-3: R6
Pollen: During pollen shed (R1)
Grain: R6/maturity

[2]Protein levels are expressed as ng/mg dry weight

[3]Minimum and maximum values were determined for each tissue across sites (n=15)
For OSR-1, DAS-59122-7 n=14 and MON 89034 x TC1507 x MON 88017 x DAS-59122-7 n=13
For OSR-2, MON 89034 x TC1507 x MON 88017 x DAS-59122-7 range does not include two samples at ND.

TABLE 4. Summary of PAT levels in corn tissues				
Maize tissue[1]	Maize line	PAT (ng/mg dwt)[2]		
		Mean	Std dev	Range[3]
OSL-1	DAS-59122-7	16.9	3.96	11.4 – 22.9
	TC1507	6.94	1.38	4.70 – 9.94
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	25.0	5.31	18.1 – 34.9
OSL-2	DAS-59122-7	14.6	3.34	9.43 – 19.6[3]
	TC1507	7.42	2.31	4.81 – 12.4
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	21.5	3.61	14.6 – 26.4
OSL-3	DAS-59122-7	19.5	3.38	13.2 – 24.3
	TC1507	7.92	2.19	5.15 – 12.3
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	26.3	4.41	17.0 – 32.4
OSR-1	DAS-59122-7	2.39	0.71	0.77 – 3.53[3]
	TC1507	0.69	0.19	0.20 – 0.90[3]
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	3.74	1.24	2.39 – 7.34[3]
OSR-2	DAS-59122-7	2.69	0.92	1.02 – 4.77
	TC1507	0.80	0.28	0.40 – 1.17
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	3.84	1.26	1.80 – 6.05[3]
OSR-3	DAS-59122-7	0.94	0.40	0.28 – 1.62
	TC1507	0.26	0.08	0.16 – 0.44
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	1.10	0.43	0.36 – 1.85
OSWP-1	DAS-59122-7	4.61	1.04	2.90 – 6.72
	TC1507	1.86	0.53	1.01 – 2.76
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	7.43	2.47	4.33 – 11.9
OSWP-2	DAS-59122-7	1.58	0.64	0.70 – 2.97
	TC1507	0.52	0.16	0.26 – 0.81
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	2.51	0.86	1.56 – 4.45
OSWP-3	DAS-59122-7	0.16	0.16	ND – 0.49
	TC1507	(0.05)	0.06	ND – 0.17
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	0.22	0.23	ND – 0.80
Pollen	DAS-59122-7	ND	NA	ND – ND
	TC1507	ND	NA	ND – ND
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	ND	NA	ND – ND
Grain	DAS-59122-7	(0.049)	0.018	(0.026) -0.091
	TC1507	ND	NA	ND – ND
	MON 89034 x TC1507 x MON 88017 x DAS-59122-7	(0.050)	0.023	ND – 0.10

Data from pp. 31-32, MRID 47444907

[1]Tissues collected at the following approximate growth stages (Ritchie et al., 1997):
OSL-1: V2-V4; OSL-2: V8-V10; OSL-3: R1
OSR-1: V2-V4; OSR-2: V5-V6; OSR-3: R1
OSWP-1: V10-V12; OSWP-2: R4-R5; OSWP-3: R6
Pollen: During pollen shed (R1)
Grain: R6/maturity

[2]Protein levels are expressed as ng/mg dry weight

[3]Minimum and maximum values were determined for each tissue across sites (n=15)
For OSL-2, DAS-59122-7 range does not include one sample at ND
For OSR-1, DAS-59122-7 and TC1507 n=14, and MON 89034 x TC1507 x MON 88017 x DAS-59122-7 n=13
For OSR-2, MON 89034 x TC1507 x MON 88017 x DAS-59122-7 range does not include one sample at ND.
Values in parentheses are between the method LOD and LOQ.

Study Authors' Conclusions

The study authors concluded that, for TC1507, the maximum protein levels in leaf, root, whole plant, pollen and grain were 34, 13, 13, 23, and 4.6 ng/mg dwt, respectively, for Cry1F; and 12,

1.2, 2.8 ng/mg dwt, not detectable, and not detectable, respectively, for PAT. For DAS-59122-7, the maximum protein levels were 250, 150, 230, 98, and 100 ng/mg dwt, respectively, for Cry34Ab1; 170, 81, 81, 0.06, and 2.7 ng/mg dwt, respectively for Cry35Ab1; and 24, 4.8, 6.7 ng/mg dwt, not detectable, and 0.09 ng/mg dwt, respectively, for PAT. For MON 89034 x TC1507 x MON 88017 x DAS-59122-7, the maximum protein levels were 280, 150, 200, 120, and 94 ng/mg dwt, respectively, for Cry34Ab1; 16, 71, 82, 0.53, and 2.3 ng/mg dwt, respectively, for Cry35Ab1; 31, 15, 16, 32, and 7.4 ng/mg dwt, respectively, for Cry1F; and 35, 7.3, 12 ng/mg dwt, not detectable, and 0.10 ng/mg dwt, respectively, for PAT.

Reviewer's Conclusion

The reviewer agrees with the study authors' conclusions.

References

Ritchie, S.W., J.J. Hanway, and G.O. Benson. 1997. How a Corn Plant Develops: Special Report #48. Iowa State University of Science and Technology Cooperative Extension Service, Ames, Iowa