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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MAR 18 2009

MEMORANDUM

SUBJECT: Environmental Risk Assessment for MON 89034 x TC1507 x MON 88017 x DAS-59122-7 Combined Insecticidal Trait Corn Product [EPA Reg. No. 524-LIR] in support of a FIFRA Sec. 3 Registration; Decision No. 394799; DP Barcode: 3355690; Submission No. 830991; submitted by Monsanto Co.

FROM: Shannon Borges, Biologist
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division, 7511P

THROUGH: Zigfridas Vaituzis, Ph.D., Senior Scientist
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 REQUEST: To review the environmental fate and effects data in support for Sec. 3 Registration for MON 89034 x TC1507 x MON 88017 x DAS-59122-7 combined PIP corn product.

CONCLUSION:
At present, the Agency has not identified any significant adverse effects of the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, or Cry34/35Ab1 proteins on the abundance of non-target organisms in any field population, whether expressed individually or as MON 89034 x TC1507 x MON 88017 x DAS-59122-7 combined PIP corn product. The potential for synergistic effects has been evaluated and the data that were reviewed for the individual parental events can be bridged to support the Sec. 3 registration of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 combined PIP corn product.

It is unlikely that direct or indirect harmful effects to non-target organisms, including federally-listed threatened or endangered species, would result from the insecticidal proteins Cry1A.105,

Cry2Ab2, Cry1F, Cry3Bb1, or Cry34/35Ab as a result of the proposed Sec. 3 registration. The Agency anticipates that for full commercial cultivation, no hazard will result to the environment.

I. Environmental Risk Assessment for MON 89034 x TC1507 x MON 88017 x DAS-59122-7 combined PIP corn product

SUMMARY

Monsanto Company has submitted an application and is seeking Sec. 3 registration for MON 89034 x TC1507 x MON 88017 x DAS-59122-7 [EPA Reg No. 524-LIR] as expressed in transgenic corn (*Zea mays*). This combined PIP product was developed by crosses (via traditional breeding methods) from each of its individual PIP events.

Event MON 89034 produces the Cry1A.105 and Cry2Ab2 Bt proteins, and Event TC1507 produces Cry1F. These proteins are intended to control several lepidopteran pests of corn, including European corn borer (ECB, *Ostrinia nubilalis*), corn ear worm (CEW, *Helicoverpa zea*), fall army worm (FAW, *Spodoptera frugiperda*), and black cutworm (BCW, *Agrotis ipsilon*). Event 88017 produces the Cry3Bb1 protein, and Event DAS-59122-7 produces the binary protein Cry34/35Ab1. These two events provide additional control for coleopteran pests, particularly corn rootworm pests (*Diabrotica* spp.).

It has been determined that each individual event has protein expression levels that are comparable to the MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn hybrid (Kough, 2009). Therefore, the margins of exposure that were previously determined for the insecticidal proteins in the individual events can be cited for the risk assessments of these proteins in the stacked hybrid. Additionally, no synergistic or antagonistic effects were observed in several combinations of the individual events in MON 89034 x TC1507 x MON 88017 x DAS-59122-7, as well as the MON 89034 x TC1507 x MON 88017 x DAS-59122-7 hybrid itself. As a result, the Agency concludes that there is no indication of synergistic effects or increased levels of protein expressed in the combined PIP product, so the environmental risk assessment for the single PIP lines are applicable to the assessment of MON 89034 x TC1507 x MON 88017 x DAS-59122-7.

As a result, the environmental risk assessment of the individual events, as well as an additional study submitted on the toxicity of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 to a non-target insect, the Agency concludes that there will be no unreasonable adverse effects to the environment, including endangered species, by MON 89034 x TC1507 x MON 88017 x DAS-59122-7 combined trait corn.

BACKGROUND

Monsanto Company is applying for a FIFRA Section 3 Registration for the combined trait corn product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 that confers insect resistance and herbicide tolerance. The data to support this registration are based on data submitted for the individual parental lines (including corn events TC1507, MON 88017, DAS-59122-7) that were

previously reviewed and registered for full commercial cultivation by the Agency. Synergism studies involving the Cry1A.105, Cry2Ab2, and Cry3Bb1 proteins were previously submitted and have been reviewed by the Agency. Two additional synergism studies with susceptible lepidopteran and coleopteran species exposed either to subsets or all of the Cry proteins in MON 89034 × TC1507 × MON 88017 × DAS-59122-7 have been submitted and reviewed by the Agency. These studies provide support for bridging to data for the individual PIP events that are already contained in the Agency's database.

The combined trait corn MON 89034 x TC1507 x MON 88017 x DAS-59122-7 product was developed by crosses (via traditional breeding methods) from the following individual events:

1. **MON 89034** produces two insecticidal proteins that protect against feeding damage caused by European corn borer (*Ostrinia nubilalis*) and other lepidopteran insect pests. MON 89034 produces two *Bacillus thuringiensis* proteins, Cry2Ab2 (subsp. *kurstaki*) protein and Cry1A.105, a modified Cry1A *Bt* protein. The ecological effects and environmental fate of Event MON 89034 corn were reviewed by the Agency [see EPA memoranda: Hunter and Vaituzis, 2006; Milofsky and Vaituzis, 2006; and Vaituzis, 2008].

2. **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. A summary of the Agency's environmental risk assessment for Cry1F corn is found in the Agency's *Bt* Crops Reassessment (EPA, 2001a) and updated in the 2001 Biopesticides Registration Action Document for Cry1F (EPA, 2001b).

3. **MON 88017** produces a modified *Bacillus thuringiensis* (subsp. *Kumamotoensis*) Cry3Bb1 protein to protect against corn rootworm (CRW) larval feeding. The Agency's environmental risk assessment is found in the updated 2005 Biopesticides Registration Action Document for Cry3Bb1 (EPA, 2005a).

4. **DAS-59122-7** (Herculex RW™, DAS-59122-7) produces the *Bacillus thuringiensis* strain PS149B1 Cry34Ab1 and Cry35Ab1 proteins to protect against coleopteran pests such as corn rootworm. The Agency's environmental risk assessment is found in the 2005 Biopesticides Registration Action Document for Cry34Ab1 and Cry35Ab1 (EPA, 2005b).

ENVIRONMENTAL ASSESSMENT

An ecological risk assessment was conducted to characterize the risk of adverse impacts of the traits expressed in combined trait MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn product on non-target organisms, particularly invertebrates found in or near agro-ecosystems. Concerns over possible effects on endangered and threatened species, especially butterfly (Lepidoptera) and beetle (Coleoptera) species have also been addressed.

The hazard of each of the individual events in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 has previously been addressed, and the previous assessments are summarized below. A unique concern with the environmental risk assessment of stacked PIPs is the potential for synergistic effects that may result from interaction between the Cry proteins contained in the

hybrid plants. Therefore, this assessment additionally contains an evaluation of the potential for synergism in the MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn hybrid. This was determined through studies that were previously submitted to the Agency for other registrations involving the parent PIP events, as well as two additional studies specifically submitted for this proposed registration.

A. Event MON 89034 (lepidopteran active) Environmental Risk Assessment

Potential adverse effects to non-target organisms by the Cry1A.105 and Cry2Ab2 proteins in expressed by event MON 89034 have been reviewed (Vaituzis 2008). The following is a summary of the MON 89034 environmental risk assessment.

Both the Cry1A.105 and Cry2Ab2 proteins are intended to control lepidopteran pests of corn. The toxicity of these proteins was previously reviewed for the Section 3 registration of the MON 89034 x MON 88017 combined trait corn product. In this assessment, the hazard of these proteins was evaluated for the lady beetle, minute pirate bug, parasitic hymenoptera, collembolan, *Daphnia*, honey bee, earthworm, and birds, mammals, fish, and non-target plants. Reproductive and developmental observations were also made in the lady beetle, minute pirate bug and honeybee studies. A soil degradation/persistence study was also evaluated. With the exception of the *Daphnia* study, these studies were found to be acceptable (an additional *Daphnia* study was required as a condition of registration). Interaction between the Cry1A.105 and Cry2Ab2 proteins was reviewed in the assessment for the MON 89034 x MON 88017 Experimental Use Permit, which provided evidence that these proteins did not interact. Additionally, gene flow and weediness assessments via pollen and Cry protein DNA uptake by plants and soil microorganisms previously have been performed. Based on this information, EPA concluded that risk to non-target wildlife, aquatic, and soil organisms is not expected from Cry1A.105 or Cry2Ab2 expressed in MON 89034.

At present, the Agency is aware of no identified significant adverse effects of Cry1A.105 or Cry2Ab2 on the abundance of non-target organisms in any population in the aquatic or terrestrial field environment, whether they are animals, plants, pest parasites, pest predators, or pollinators. Further, the EPA believes that cultivation of MON 89034 corn may have fewer adverse impacts on non-target organisms than use of chemical pesticides for corn production, because under normal circumstances, MON 89034 corn requires substantially fewer applications of chemical pesticides, compared to production of non-*Bt* corn. Fewer chemical insecticide applications generally result in increased populations of beneficial organisms that control secondary pests, such as aphids and leafhoppers. In addition, no adverse effect on Federally listed endangered and threatened species is expected. Further, the EPA has determined that there is no significant risk of gene capture and expression of Cry1A.105 or Cry2b2 proteins by wild or weedy relatives of corn in the U.S., its possessions, or territories, available data do not indicate that Cry proteins have any measurable adverse effect on microbial populations in the soil, nor has horizontal transfer of genes from transgenic plants to soil bacteria been demonstrated.

In conclusion, the risk assessment found no hazard to the environment from cultivation of Event MON 89034 corn expressing Cry1A.105 and Cry2Ab2.

4

B. Event TC1507 (lepidopteran active) Environmental Risk Assessment

Potential adverse effects to non-target organisms by Cry1F protein has been reviewed in the Agency's *Bt* Crops Reassessment (EPA, 2001a) and in the updated Biopesticides Registration Action Document for Cry1F (EPA, 2004). The following is a summary of the Cry1F environmental risk assessment. Prior to the registration of Cry1F in 2004, EPA performed risk assessments on plants, wild mammals, birds, fish, aquatic invertebrates, earthworms, terrestrial non-target insects (including honey bee, parasitic wasps, green lacewings, ladybird beetle, springtails [Collembola toxicity/reproduction], and monarch butterflies), as well as field evaluations of the effects of Cry1F exposure on non-target insects in corn fields, soil degradation/persistence studies, and an endangered species impact assessment, particularly for Lepidoptera. In addition, gene flow and weediness assessments via pollen and Cry protein DNA uptake by plants were also performed. Cry1F protein in soil has been shown to degrade rapidly to very low levels. EPA concluded that there is sufficient information to believe that there is no risk from the uses of Cry1F corn to non-target wildlife, aquatic, and soil organisms.
At present, the Agency is aware of no identified significant adverse effects of Cry1F protein on the abundance of non-target organisms in any population in the aquatic or terrestrial field environment, whether they are animals, plants, pest parasites, pest predators, or pollinators. Field testing and field census data submitted to the Agency show minimal to undetectable changes in the beneficial insect abundance or diversity. To date the available field test data show that compared to crops treated with conventional chemical pesticides, the transgenic crops have no detrimental effect on the abundance of non-target invertebrate populations.

The EPA has reviewed the potential for gene capture and expression of Cry1F protein by wild or weedy relatives of corn in the United States, its possessions or territories and has found that there is no significant risk in the United States, its possessions or territories (EPA 2001a). Domesticated corn does not have a reasonable possibility of passing its traits to wild maize species. Feral species related to corn (within the United States) cannot be pollinated due to differences in chromosome number, phenology (periodicity or timing of events within an organism's life cycle as related to climate, e.g., flowering time) and habitat. Concern over species related to maize (*Zea mays* ssp. *mays*), such as *Tripsacum* species and the teosintes, as potential recipients of gene flow from genetically modified Zea mays spurred the EPA to take a closer look at this topic (EPA 2001a and b). Upon review, the EPA found that any introgression of genes into wild teosinte from *Zea mays* was not considered to be a significant agricultural or environmental risk. Furthermore, the Agency stated that the growth habits of teosintes are such that the potential for serious weedy propagation and development is not biologically plausible in the United States.

In conclusion, the risk assessment found no hazard to the environment from cultivation of Event TC1507 corn expressing Cry1F protein.

C. MON 88017 (coleopteran active) Environmental Risk Assessment

Potential adverse effects to non-target organisms by Cry3Bb1 protein has been reviewed (EPA 2003). The following is a summary of the Cry3Bb1 environmental risk assessment.

For registration of Cry3Bb1, EPA reviewed studies conducted on representative non-target species with several Cry3Bb1 protein variants and performed risk assessments on plants, wild mammals, birds, fish, aquatic invertebrates, estuarine and marine animals, earthworms, terrestrial non-target insects including honey bee adults and larvae, parasitic wasps, green lacewings, several lady beetle species, springtails (Collembola toxicity/reproduction), monarch butterflies, field evaluations of the effects of Cry3Bb1 exposure on non-target invertebrates, soil degradation/persistence studies and an endangered species impact assessment (EPA, 2003). In addition, gene flow and weediness assessments via pollen and Cry protein DNA uptake by plants and soil microorganisms were also performed. EPA concluded that the Agency has sufficient information to believe that there is no risk from the uses of Cry3Bb1 corn to non-target wildlife, aquatic, and soil organisms.

In 2007 an additional assessment of possible effects on Hungerford's crawling water beetle was performed. Hungerford's crawling water beetle species is currently known to occur in only six streams - five in mostly northern Michigan and one in Ontario, Canada. These are not major corn growing areas. The beetles are found in the cool riffles of clean, slightly alkaline streams. All streams where this beetle has been found have moderate to fast water flow, good stream aeration, inorganic substrate, with an open to partially open canopy just below beaver dams or similar human-made structures. Adults prefer gravel and cobble riffles while larvae occupy areas with slower current and dense growth of microalgae, especially *Chara*. Since the Hungerford's crawling water beetle larvae are reported to feed on filamentous algae (and possibly periphytic diatoms), no dietary exposure to anti-coleopteran Cry protein in corn tissue is expected. Therefore, the previous finding of No Effect (NE), direct or indirect, from cultivation of anti-coleopteran Cry protein containing corn to Hungerford's crawling water is confirmed.

At present, the Agency is aware of no identified significant adverse effects of Cry protein on the abundance of non-target organisms in any population in the aquatic or terrestrial field environment, whether they are animals, plants, pest parasites, pest predators, or pollinators. Field testing and field census data submitted to the Agency show minimal to undetectable changes in the beneficial insect abundance or diversity. In corn fields densities of predatory and non-target insects are generally higher on Cry3Bb1 corn than non-Bt corn. Two year invertebrate abundance studies do not show a shift in biodiversity in Cry3Bb1 corn fields, except in cases where the predators are dependent on the pest insect as prey. In contrast, treatment with chemical pesticides, when studied, had significant effects on the total numbers of insects and on the numbers within the specific groups. To date the available field test data show that compared to crops treated with conventional chemical pesticides, the transgenic crops have no detrimental effect on the abundance of non-target invertebrate populations.

The movement of transgenes from Cry3Bb1 host plant into weeds and other crops has also been considered. The Agency has determined that there is no significant risk of gene capture and expression of Cry3Bb1 protein by wild or weedy relatives of corn in the U.S., its possessions or territories. The fate of Cry3Bb1 protein in soils and indirect effects on soil biota have also been evaluated (EPA, 2003; Icoz and Stotzky, 2007). The data show that most of the Cry protein deposited into soil is quickly degraded, although a residual amount may persist in biologically active form for a much longer period of time. It is also reported that the same degree of Bt Cry protein persistence takes place in soils that have been exposed to repeat Bt spray applications

when compared to soil exposed to growing Bt crop. Limited data do not indicate that Cry proteins have any measurable effect on microbial populations in the soil. Horizontal transfer from transgenic plants to soil bacteria has not been demonstrated (Sanvido, et al.2007). Published studies of Bt Cry protein in soil show no effect on bacteria, actinomyces, fungi, protozoa, algae, nematodes, springtails or earthworms (Saxena and Stotzky, 2001). In addition, new plants grown in Bt Cry protein-containing soil do not take up the Bt protein.

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

D. Event DAS-59122-7 (coleopteran active) Environmental Risk Assessment

Potential adverse effects to non-target organisms by Cry34/35Ab1 protein has been reviewed in the Agency's Biopesticides Registration Action Document for *Bt* Cry34Ab1 and Cry35Ab1 Proteins (EPA, 2005b). The following is a summary of the Cry34/35Ab1 environmental risk assessment.

For registration of Cry34Ab1 and Cry35Ab1 proteins, EPA reviewed studies conducted on representative non-target species and performed risk assessments on plants, wild mammals, birds, freshwater fish, freshwater aquatic invertebrates, estuarine and marine animals, earthworms, terrestrial non-target insects including honey bee, parasitic wasp, green lacewing, lady beetle, and springtails (Collembola toxicity/reproduction). Data were also submitted on the insecticidal activity spectrum and soil degradation/persistence studies, and field monitoring of nontarget terrestrial invertebrates, and an endangered species impact assessment and nontarget invertebrate impact assessment were also submitted. In addition, the potential for horizontal gene transfer, gene flow, and weediness was assessed. EPA concluded that based on the required and voluntarily developed test data on Cry34/35Ab1 corn, the levels of Cry34/35Ab1 protein in corn will not pose unreasonable adverse effects to corn field flora and fauna. Available data also indicate that there should be minimal short-term accumulation of Cry34/35Ab1 protein in agricultural soil. In addition, no adverse effect on endangered and threatened species listed by the US Fish and Wildlife Service is expected from the registration of Cry34/35Ab1 corn.

At present, the Agency is aware of no identified significant adverse effects of Cry34/35Ab1 proteins on the abundance of non-target beneficial organisms in any population in the field, whether they are pest parasites, pest predators, or pollinators. Field testing and field census data submitted to the Agency show minimal to undetectable changes in the beneficial insect abundance or diversity. To date, available field test data show that compared to crops treated with conventional chemical pesticides, the transgenic crops have no detrimental effect on the abundance of non-target insect populations. The Agency believes that cultivation of Cry34/35Ab1 corn may result in fewer adverse impacts to non-target organisms than result from the use of chemical pesticides. Under normal circumstances, Cry34/35Ab1 corn requires substantially fewer applications of chemical pesticides. This should result in fewer adverse impacts to non-target organisms because application of nonspecific conventional chemical pesticides is known to have an adverse effect on non-target beneficial organisms found living in the complex environment of an agricultural field. Many of these beneficial organisms are important integrated pest management controls (IPM) for secondary pests such as aphids and leafhoppers. The overall result

of cultivation of corn expressing Cry34/35Ab1 proteins is that the number of chemical insecticide applications for non-target pest control is reduced for management of multiple pest problems.

The movement of transgenes from Cry34/35Ab1 host plants into weeds and other crops has also been considered. The Agency has determined that there is no significant risk of gene capture and expression of Cry34/35Ab1 protein by wild or weedy relatives of corn in the U.S., its possessions, or territories. The fate of Cry34/35Ab1 protein in soils and indirect effects on soil biota have also been evaluated. Test data show that most of the Cry protein deposited into soil is quickly degraded, although a residual amount may persist in biologically active form for a much longer period of time. It is also reported that the same degree of Bt Cry protein persistence takes place in soils that have been exposed to repeat Bt spray applications when compared to soil exposed to growing Bt crop. Limited data do not indicate that Cry proteins have any measurable effect on microbial populations in the soil. Horizontal transfer of genes from transgenic plants to soil bacteria has not been demonstrated. Published studies of Bt Cry protein in soil show no effect on bacteria, actinomyces, fungi, protozoa, algae, nematodes, springtails or earthworms. In addition, new plants planted in Bt Cry protein containing soil do not take up the Bt protein.
This risk assessment finds no hazard to the environment at the present time from cultivation of Cry34/35Ab1 protein expressing corn.

E. Synergism studies

The purpose of these studies was to characterize the potential for interaction between the Bt Cry proteins contained in combined trait corn event MON 89034 x TC1507 x MON 88017 x DAS-59122-7. In order to bridge the ecological effects and environmental fate data of the individual parental events to the combined PIP products, the effects of the pesticidal mixture of the combined PIP product must be tested on a susceptible pest species, via diet-incorporation bioassays. Interactions between the test materials can be assessed by comparing the larval mortality observed for the mixed proteins with the predicted responses based on the bioassay of each protein individually. If there is no greater mortality than expected over the range of concentrations in a sensitive pest species, it is likely that there will be no synergism of the mixture against non-target organisms.

i. Potential interaction between Cry1A.105, CryA2b2 and Cry3Bb1 proteins (MRID 469513-05 & 469513-06)

The purpose of these studies was to characterize the potential for interaction between the lepidopteran-active proteins Cry1A.105 and Cry2Ab2 and the coleopteran-active protein Cry3Bb1. The Cry1A.105 and Cry1A.105 and Cry2Ab2 proteins were tested alone and in combination with either the Cry3Bb1 protein against European corn borer (ECB, *Ostrinia nubilalis*) and corn ear worm (CEW, *Helicoverpa zea*) in diet incorporation studies. Likewise, the Cry3Bb1 protein was tested alone and with the Cry1A.105 and/or the Cry2Ab2 proteins, against the Colorado potato beetle (CPB, *Leptinotarsa decemlineata*). The activity of Cry1A.105 and Cry2Ab2 proteins was not significantly altered by the presence of Cry3Bb1, and the activity of Cry3Bb1 was not significantly altered by the presence of Cry1A.105 and/or Cry2Ab2. Collectively these data provide evidence that the proteins do not interact in an antagonistic, additive, or synergistic manner. This study, along with the interaction study between Cry1A.105

and Cry2Ab2 reviewed for the MON 89034 Experimental Use Permit indicate that MON 89034 x MON 88017 maize will not result in any unexpected interaction in an antagonistic, additive, or synergistic manner with regard to target insects. It is therefore extremely unlikely that the Cry1A.105, Cry2Ab2 and Cry3Bb1 proteins contained in a single plant will impart any hazard to non-target organisms exposed to these hybrids in the environment.

ii. Potential interactions between lepidopteran active proteins (Cry1A.105, Cry2Ab2, and Cry1F), coleopteran active proteins (Cry3Bb1 and Cry34/35Ab1), and between lepidopteran active and coleopteran active proteins

The purpose of these studies was to test the potential for interactions among the subsets of the proteins expressed by combined trait corn event MON 89034 x TC1507 x MON 88017 x DAS-59122-7 that had not previously been tested. Each study is summarized below.

MRID No. 474449-10

This study was performed to evaluate the potential for interactions among the proteins produced by MON 89034 x TC1507 x MON 88017 x DAS-59122-7, specifically 1) interactions among lepidopteran-active proteins (Cry1A.105, Cry2Ab2, and Cry1F), and 2) interactions between lepidopteran and coleopteran (Cry3Bb1 and Cry34/35Ab1) proteins. MON 89034 x TC1507 x NK603 (a trait conferring glyphosate resistance) was used to assess the potential for interaction between the lepidopteran proteins. European corn borer (ECB, *Ostrinia nubilalis*) larvae were exposed to a series of lyophilized leaf tissue concentrations of either MON 89034, TC1507, or MON 89034 x TC1507 x NK603 in diet incorporation assays run in parallel. The GI_{50} values (50% growth inhibition) were estimated from these assays for comparison to predicted values based on the GI_{50} for each individual component event. Predicted values were based on a concentration addition model (Finney, 1971; Tabashnik, 1992). In a concurrent assay, ECB were exposed to a series of lyophilized leaf tissue concentrations of either MON 89034 x TC1507 x NK603 or MON 89034 x TC1507 x MON 88017 x DAS-59122-7 to determine whether combined Cry1A.105, Cry2Ab2, and Cry1F activity is altered by the presence of Cry3Bb1 and Cry34/35Ab1. The estimated GI_{50} values from MON 89034 x TC1507 x NK603 and MON 89034 x TC1507 x MON 88017 x DAS-59122-7 were then compared statistically. Control treatments included a diet-only assay control, lyophilized conventional corn tissue, lyophilized MON 88017 tissue, and lyophilized DAS-59122-7 tissue. The mean weight of ECB larvae decreased with increasing tissue concentration of each test corn product as expected. There was no significant difference between the estimated and predicted GI_{50} values for MON 89034 x TC1507 x NK603, indicating there was no interaction between the combination of the Cry1A.105 and Cry2Ab2 proteins and the Cry1F protein. The GI_{50} values for MON 89034 x TC1507 x NK603 and MON 89034 x TC1507 x MON 88017 x DAS-59122-7 also were not significantly different, indicating that the Cry3Bb1 and Cry34/35Ab1 proteins do not interact with the combined activity of the Cry1A.105, Cry2Ab2, and Cry1F proteins. These results indicate that there is no interaction among the *Bt* proteins expressed in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn.

MRID No. 474449-09

The study was conducted to assess the potential for interaction between Cry3Bb1 and Cry34Ab1/Cry35Ab1 proteins. The test organisms were neonate (<24 hours old) southern corn rootworms (SCRW, *Diabrotica undecimpunctata howardi*). Each bioassay contained seven concentrations each of Cry3Bb1 and Cry34Ab1/Cry35Ab1 as well as a third treatment consisting of a mixture of Cry3Bb1 + Cry34/35Ab1. Percent mortality and growth inhibition, based on reduction in live weight, were calculated. Predicted LC_{50} and GI_{50} values for the combination of all three proteins were determined based on the concentration addition model (Finney 1971, Tabashnik 1992). The predicted LC_{50} and GI_{50} values for Cry3Bb1 + Cry34/35Ab1 were not significantly different from the observed LC_{50} and GI_{50} values, indicating there is no evidence of interaction (synergism or antagonism) between Cry3Bb1 and the Cry34Ab1 and Cry35Ab1 proteins.

MRID 474449-08

Monsanto Co. provided a discussion of the results of the above studies, along with information from the public literature, to support the assessment of interaction. This study did provide some supporting evidence from the literature based generally on receptor specificity of Cry proteins; however, the Agency determined that the synergism studies submitted by the registrant provide adequate evidence of no interaction.

Conclusions/Recommendations: The results of these interaction studies of the proteins contained in the combined trait corn event MON 89034 x TC1507 x MON 88017 x DAS-59122-7 indicate that there is no change in the level of activity among susceptible insects when exposed to the MON 89034 x TC1507 x MON 88017 x DAS-59122-7 hybrid or subsets of its individual events. Collectively these data provide evidence that Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, and Cry34/35Ab1 do not interact in an antagonistic or synergistic manner.

F. Effects of Combined PIP product on Non-target Organisms

The potential for interaction among the proteins in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn was tested on the pink-spotted ladybeetle (*Coleomegilla maculate*; MRID 474449-13), which is related to the target pest of Cry3Bb1 and Cry34/35Ab1. A 16-day test was conducted to determine the dietary effects of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn pollen on survival and development of the ladybird beetle (*Coleomegilla maculata*). Larvae were fed prepared diets containing the test pollen, control pollen from a conventional corn, or the control pollen plus a reference control of potassium arsenate. There was no significant difference in the development of larvae to adults between the test diet and control diet groups. No larvae fed the reference diet developed to adults. There was no significant difference in the adult biomass of the test diet and control diet groups. Survival of larvae fed the reference substance was significantly lower than that of the other two groups, confirming the validity of the test methods. Based on the data presented, exposure to MON 89034 x TC1507 x MON 88017 x DAS-59122-7 pollen at the 50% target concentration in the diet (concentrations actually ranged from 66 – 97%) did not result in adverse effects on *C. maculata*. The synergism study conclusions confirm that the six Cry proteins in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 stacked and

10

produced in one plant do not pose any hazard to the ladybird beetle (*C. maculata*), a representative beneficial Coleopteran species.

G. Overall Synergism Conclusion:

Collectively, the synergism studies along with the non-target organism toxicity test with *Coleomegilla maculata*, indicate that the combined PIP product MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn will not result in any unexpected interaction related to an antagonistic or synergistic action to target and non-target insects. Additionally, no systematic increase was observed in expression of Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, and Cry34/35Ab1 proteins in the combined PIP product compared to the individual events (Kough, 2009). Therefore, increased exposure to each of these proteins is also not expected. This information, the compilation of ecotoxicity studies on non-target organisms, and efficacy and field data support the bridging of the environmental risk assessment from the original parental events to the combined PIP MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn product.

H. Threatened and Endangered Species Considerations

The potential risk to listed threatened or endangered species has been previously addressed for Bt Cry proteins in corn (Vaituzis, 2008, EPA, 2001a and b, EPA, 2005a and b) and was addressed specifically for MON 89034 x TC1507 x MON 88017 x DAS-59122-7 in the EUP risk assessment for this combined trait corn product (Waggoner and Vaituzis 2008). A brief summary of the findings in these documents is presented below. Monsanto Co. also submitted an endangered species assessment (MRID 474449-12), which was reviewed and found to be consistent with the Agency's conclusions.

The primary route of exposure to Cry1A.105 and Cry2Ab2 proteins in corn is through ingestion of corn tissue. Since Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, and Cry34/35Ab1 proteins have not been shown to have toxic effects on mammals, birds, plants, aquatic species, insects and other invertebrate species at the Estimated Environmental Concentration (EEC), a "may affect" situation for endangered land and aquatic species is not anticipated. In addition, EPA does not expect that any threatened or endangered plant species will be affected by outcrossing to wild relatives or by competition with such entities. Hybrid corn does not exist in the wild, nor are there wild plants that can interbreed with corn in the United States.

Because of the selectivity of Cry1A.105, Cry2Ab2, and Cry1F proteins for lepidopteran species and of Cry3Bb1 and Cry34/35Ab1 proteins for coleopteran species, endangered species concerns are mainly restricted to these two orders. Examination of data showing the county level distribution of endangered/threatened lepidopteran and coleopteran species (currently listed by the U.S. Fish and Wildlife Service) relative to corn production counties in the United States clearly indicated that any potential concern regarding range overlap with corn production was mainly restricted to the Karner blue butterfly (*Lycaeides melissa samuelis*) and the American burying beetle (*Nicrophorus americanus*). It has been previously determined that it is unlikely that sufficient pollen expressing Cry protein would accumulate on the wild lupine (*Lupinus perennis*), which constitutes the Karner blue butterfly's sole food source. Therefore, exposure to this species is not expected. While overlap in habitat of the American burying beetle may occur, the larvae

and adult beetles feed exclusively on carrion with some limited adult predation. Therefore, even if populations of American burying beetles did occur in proximity to *Bt* corn fields, there would be little chance of exposure to the Cry proteins due to their feeding habits. Concerns of potential exposure to Hungerford's crawling water beetle (*Brychius hungerfordi*) had also been previously explored by the Agency and are discussed above; the Agency determined that there would be "No Effect" to this species.

In addition, the Agency previously determined there are no sexually compatible wild or weedy relatives of maize (*Zea mays*) in the United States or its territories (EPA, 2001a). Thereby, genes containing Cry protein endotoxins cannot escape into plants on which endangered or threatened species feed on in these areas.

In conclusion, there will be No Effect (NE), direct or indirect, on endangered and threatened species or their habitat as listed by the United States Fish and Wildlife Service (USFWS) and the National Marine Fisheries Services (NMFS), including mammals, birds or terrestrial and aquatic plants and invertebrate species as a result of cultivation of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn plants. Therefore, no consultation with the USFWS is required under the Endangered Species Act.

I. Supplemental data needed to confirm MON 89034 x TC1507 x MON 88017 x DAS-59122-7 Non-Target Hazard Assessment

The Agency has sufficient information to believe that there is no risk from the proposed uses of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn to non-target wildlife, aquatic, and soil organisms. In previous Section 3 registrations of the individual PIP events in MON 89034 x TC1507 x MON 88017 x DAS-59122-7, the Agency required registrants to conduct studies as a condition of registration. Therefore, a condition of registration for the MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn stacked product is based on the registrant's data submission to satisfy the conditions of registration for each of the parental events.

CONCLUSION

The environmental risk assessment indicates for the MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn hybrid, based on prior assessments conducted on Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, and Cry34/35Ab1 proteins individually, that no unreasonable harm will result to the environment or any federally-listed threatened or endangered species from commercial cultivation of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn hybrids. The Agency has determined that MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn hybrids will have No Effect (NE) on endangered and/or threatened species listed by the US Fish and Wildlife Service (USFWS) and the National Marine Fisheries Services (NMFS), including mammals, birds, terrestrial and aquatic plants, and invertebrate species. Therefore, no consultation with the USFWS is required under the Endangered Species Act.

The Agency believes that cultivation of MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn hybrids may result in fewer adverse impacts to non-target organisms than result from the use of chemical pesticides. Under normal circumstances, *Bt* corn requires substantially fewer

applications of chemical pesticides. This should result in fewer adverse impacts to non-target organisms because application of nonspecific conventional chemical pesticides is known to have an adverse effect on non-target beneficial organisms found living in the complex environment of an agricultural field. Many of these beneficial organisms are important integrated pest management controls (IPM) for secondary pests such as aphids and leafhoppers. Therefore, the overall result of cultivation MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn hybrids, expressing Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, and Cry34/35Ab1 proteins, is that the number of chemical insecticide applications for non-target pest control will be reduced for management of multiple pest problems.

REFERENCES

EPA (2001a). Biopesticides Registration Action Document for *Bacillus thuringiensis* (*Bt*) Plant-Incorporated Protectants, dated October 21, 2001. US Environmental Protection Agency, Washington, DC.

EPA (2001b). Biopesticides Registration Action Document for *Bacillus thuringiensis* (*Bt*) Cry1F Plant-Incorporated Protectants, updated August 2005. US Environmental Protection Agency, Washington, D.C.

EPA. (2003). Ecological hazard assessment for *Bacillus thuringiensis* Cry3Bb1 protein, EPA Reg. No. 524-LEI.

EPA (2004). Biopesticides Registration Action Document for *Bacillus thuringiensis* Cry1F corn, updated August 2005. US Environmental Protection Agency, Washington, D.C.

EPA (2005a). Biopesticides Registration Action Document for *Bacillus thuringiensis* (*Bt*) Cry3Bb1 Plant-Incorporated Protectants, updated June 2007. US Environmental Protection Agency, Washington, D.C.

EPA (2005b). Biopesticides Registration Action Document for *Bacillus thuringiensis* (*Bt*) Cry34Ab1 and Cry35Ab2 Plant-Incorporated Protectants, dated October 2005. US Environmental Protection Agency, Washington, D.C.

Finney, D.J. 1971. Probit Analysis. Cambridge University Press, London.

Hunter, M. and Z. Vaituzis (2006). Review of Non-Target Invertebrate Studies for MON 89034 Maize Containing Cry1A.105 and Cry2Ab2 Proteins EUP (524-LTL). U.S. EPA. Washington, D.C.

Icoz, I, and G. Stotzky (2007). Cry3Bb1 protein from *Bacillus thuringiensis* in root exudates and biomass of transgenic corn does not persist in soil. Transgenic Research, September 13, 2007.

Kough, J. (2009). BPPD Review of SmartStax Plant-Incorporated Protectant (PIP). U.S. EPA, Washington, D.C. Memorandum dated March 13, 2009.

Milofsky, T. and Z. Vaituzis (2006). Environmental Risk Assessment for Monsanto's MON89034 x MON88017 *Bacillus thuringiensis* Corn EUP. U.S.EPA, Washington, D.C.

Sanvido,O., Romeis, J., Bigler, F. (2007). Ecological Impacts of Genetically Modified Crops: Ten Years of Field Research and Commercial Cultivation. Adv Biochem Engin/Biotechnol 107: 235–278

Saxena, D. and Stotzky, G. (2001) *Bacillus thuringiensis* (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. Soil Biol. Biochem., 33, 1225–1230

Tabashnik, B. E. 1992. Evaluation of synergism among *Bacillus thuringiensis* toxins. Applied and Environmental Microbiology 58(10):3343-3346.

Vaituzis, Z. (2008). Environmental Risk Assessment for *Bacillus thuringiensis* (*Bt*) Cry1A.1015 and Cry2Ab2 insect control proteins as expressed in MON89034 corn and its associated breeding stack, MON89034 x MON88017 corn, containing *Bt* Cry3Bb1 insect control protein; submitted by Monsanto Company. U.S. EPA, Washington, D.C.

Waggoner, A. and Z. Vaituzis (2008). Environmental Risk Assessment for MON 89034 x TC1507 x MON 88017 x DAS-59122-7 Combined Insecticidal Trait Corn Product in support for an Experimental Use Permit; Biopesticides and Pollution Prevention Division. U.S. Environmental Protection Agency. Washington, D.C. Memorandum dated June 2, 2008.

DATA EVALUATION RECORD

BACILLUS THURINGIENSIS CRY1A.105 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR PV-ZM IR245) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY2AB2 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR PV-ZM IR245) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS VAR. AIZAWAI CRY1F (SYNPRO) AND THE GENETIC MATERIAL (FROM THE INSERT OF PLASMID PGMA2810) NECESSARY FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY3BB1 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR ZMI R39) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY34AB1 AND CRY35AB1 PROTEINS AND THE GENETIC MATERIAL NECESSARY FOR THEIR PRODUCTION IN CORN
(MON 89034 X TC1507 X MON 88017 X DAS-59122-7)

STUDY TYPE: Product Performance (810.3000)

MRID 47444908

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-040

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: OCT 28 2008

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: Anthony Q Armstrong
Date: OCT 28 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: OCT 28 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: OCT 28 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

Primary Reviewer: Eric B. Lewis, Oak Ridge National Laboratory

EPA Reviewer: Shannon Borges, Zigfridas Vaituzis, OPP/BPPD

STUDY TYPE:	Summary of Synergism Data (Non-guideline)
MRID NO:	47444908
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:	MSL0021267
SPONSOR:	Monsanto Company, 800 North Lindbergh Blvd., St. Louis, MO 63167
TESTING FACILITY:	Not applicable
TITLE OF REPORT:	Studies Performed to Evaluate the Potential for Interactions Among Cry Proteins Produced in MON 89034 x TC1507 x MON 88017 x DAS-59122-7
AUTHOR:	Levine, S.L.
STUDY COMPLETED:	April 16, 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 study reports.
STUDY SUMMARY:	MON 89034 x TC1507 x MON 88017 x DAS-59122-7 is a combined trait corn that expresses the lepidopteran-active Cry1A.105, Cry2Ab2, and Cry1F proteins as well as the coleopteran-active Cry3Bb1 and binary Cry34/35Ab1 proteins. Insect bioassay studies performed to assess the potential for interactions among the proteins expressed by MON 89034 x TC1507 x MON 88017 x DAS-59122-7 found that 1) the combination of Cry3Bb1 and Cry34/35Ab1 has additive activity against the southern corn rootworm (*Diabrotica undecimpunctata howardi*) pest, 2) there is no interaction between the combined Cry1A.105 and Cry2Ab2 proteins and the Cry1F protein, and 3) there is no interaction between the combined Cry1A.105, Cry2Ab2, and Cry1F proteins and the combined Cry3Bb1 and Cry34/35Ab1 proteins.
CLASSIFICATION:	**Acceptable**

16

Product Description

MON 89034 x TC1507 x MON 88017 x DAS-59122-7 is a combined trait corn that provides insect protection against lepidopteran and coleopteran insect pests and tolerance to glyphosate and glufosinate family herbicides. MON 89034 produces the lepidopteran-active Cry1A.105 and Cry2Ab2 and proteins and TC1507 produces the lepidopteran-active Cry1F protein. MON 88017 produces the coleopteran-active Cry3BBb1 protein and DAS-45122-7 produces the binary Cry34/35Ab1 proteins.

Study Summaries

There have been only a few reports of interactive effects of Cry proteins that have either decreased (antagonism) or increased (synergism) the combined insecticidal activity towards target pests (Tabashnik, 1992; Schneph et al., 1998). Research has established that the specificity of the activity of the Cry proteins is dependent on their binding to specific receptors in the insect mid-gut, greatly limiting the potential for interaction (Lambert, et al., 1996; Van Rie et al., 1989, 1990; Hofmann et al. 1988a, 1988b; OECD, 2007). The authors also provide a reference (Shimada et al., 2006) providing evidence that these specific receptors are not present in mammals.

A number of studies have been performed to assess the potential for interactions among the proteins expressed by MON 89034 x TC1507 x MON 88017 x DAS-59122-7 (Table 1.) These studies assessed the potential for interaction among the lepidopteran-active proteins, among the corn rootworm-active proteins, and between the lepidopteran and corn rootworm-active proteins.

TABLE 1. 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. 8, MRID 47444908

The potential for interaction among Cry3Bb1 and Cry34/35Ab1 was investigated in diet-overlay bioassays with southern corn rootworm (*Diabrotica undecimpunctata howardi*) (MacRae et al., 2008). Insect mortality and growth inhibition were determined after six days and the data were analyzed using a concentration addition model (Finney, 1971; Tabashnik, 1992). LC_{50} (mortality) and GI_{50} (growth inhibition) values indicated that the combination of Cry3Bb1 and Cry34/35Ab1 has additive activity against southern corn rootworm.

The potential for interaction between the combination of the Cry1A.105 and Cry2Ab2 proteins and Cry1F was examined by Levine et al. (2008). European corn borer (*Ostrinia nubilalis*) larvae were exposed to a series of lyophilized leaf tissue concentrations of either MON 89034, TC1507, or

MON 89034 x TC1507 x NK603 in seven-day diet-incorporation bioassays. The data were analyzed using a concentration addition model and not interaction was found between the combination of Cry1A.105 and Cry2Ab2 proteins and Cry1F.

The potential for interaction between the combined Cry1A.105, Cry2Ab2 and Cry1F proteins and the Cry3Bb1 and Cry34/35Ab1 proteins was determined by Levine et al. (2008). European corn borer larvae were exposed to a series of lyophilized leaf tissue concentrations from MON 89034 x TC1507 x NK603 or MON 89034 x TC1507 x MON 88017 x DAS-59122-7 in seven-day diet-incorporation bioassays. No difference in biological activity was seen, indication no interaction between the lepidopteran-active and corn rootworm-active proteins.

Study Author's Conclusions

The study author concluded that the results of these studies indicate there is no interaction among the *Bt* Cry proteins expressed in MON 89034 x TC1507 x MON 88017 x DAS-59122-7, indicating it is valid to use the environmental safety studies previously performed for registration of the individual products to support the safety assessment for MON 89034 x TC1507 x MON 88017 x DAS-59122-7.

EPA Reviewer's Conclusion

Much of this review reiterates studies that have been submitted to the Agency demonstrating the lack of interaction between *Bt* Cry proteins expressed in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 that have already been reviewed. These studies sufficiently demonstrated a lack of interaction between these proteins in target insects. The authors also cite studies from open literature indicating the specificity of each protein for certain receptors, arguing that this specificity likely plays a role in limiting interaction. While the specificity may limit interactions in some cases, many *Bt* toxins can bind to multiple receptors and one receptor can bind multiple toxins (OECD 2007). Additionally, based on the literature provided, it was apparent that the mechanism of interaction causing synergism or antagonism is not known (Schnepf et al. 1998). Nonetheless, the synergism data submitted to the Agency support the conclusion that interaction between the *Bt* Cry proteins in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 does not occur, and that an environmental risk assessment may be conducted based on the studies submitted for each individual active ingredient.

References

Finney, D.J. 1971. Probit Analysis. Cambridge University Press, London. 333 pp.

Herman, R.A. and N.P. Storer. 2004. Investigation of Potential Interaction Between Cry1F and the Binary Cry34Ab1/Cry35Ab1 Proteins. GH-C 5748, Unpublished Report of Dow AgroSciences LLC.

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.

18

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.

Lambert, B., L. Buysse, C. Decock, et al. 1996. A *Bacillus thuringiensis* Insecticidal Crystal Protein with a High Activity Against Members of the Family Noctuidae. Appl. Envir. Microbiol. 62(1):80-86.

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.

MacRae, T., C.R. Brown, and S. L. Levine. 2005. Evaluation of the Potential for Interactions Between the *Bacillus thuringiensis* Proteins Cry1A.105 and Cry2Ab2. Monsanto Report Number MSL-19859. St. Louis, MO.

MacRae, T., C. Brown, and L. Levine. 2006. Evaluation of Potential for Interactions Between the *Bacillus thuringiensis* Proteins Cry1A.105, Cry2Ab2, and Cry3Bb1. Monsanto Technical Report Number MSL-20270. St Louis, MO.

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

OECD. 2007. Consensus Document on Safety Information on Transgenic Plants Expressing *Bacillus thuringiensis*. OECD Environment, Health and Safety Publications Series on Harmonisation of Regulatory Oversight in Biotechnology. ENV/JM/MONO(2007)14.

Schnepf, E., N. Crickmore, J. Van Rie, et al. 1998. *Bacillus thuringiensis* and its Pesticidal Crystal Proteins. Microbiol. Mol. Biol. Rev. 62:775-806.

Shimada, N., K. Miyamoto, K. Kanda, et al. 2006. *Bacillus thuringiensis* Insecticidal Cry1Ab Toxin Does Not Affect the Membrane Integrity of the Mammalian Intestinal Epithelial Cell: An in vitro Study. In Vitro Cellular & Developmental Biology – Animal 42:45-49.

Tabashnik, B.E. 1992. Evaluation of Synergism Among *Bacillus thuringiensis* toxins. Applied and Environmental Microbiology 58:3343-3346.

Van Rie, J. S. Jansens, H. Hofte, et al. 1989. Specificity of *Bacillus thuringiensis* Delta-Endotoxins, Importance of Specific Receptors on the Brush Border Membrane of the Mid-Gut of Target Insects. Eur. J. Biochem. 186:239-247.

Van Rie, J. S. Jansens, H. Hofte, et al. 1990. Receptors on the Brush Border Membrane of the Insect Midgut as Determinants of the Specificity of *Bacillus thuringiensis* Delta-Endotoxins. Appl. Environ. Microbiol. 56:1378-1385.

DATA EVALUATION RECORD

BACILLUS THURINGIENSIS CRY1A.105 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR PV-ZM IR245) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY2AB2 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR PV-ZM IR245) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS VAR. AIZAWAI CRY1F (SYNPRO) AND THE GENETIC MATERIAL (FROM THE INSERT OF PLASMID PGMA2810) NECESSARY FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY3BB1 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR ZMI R39) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY34AB1 AND CRY35AB1 PROTEINS AND THE GENETIC MATERIAL NECESSARY FOR THEIR PRODUCTION IN CORN
(MON 89034 X TC1507 X MON 88017 X DAS-59122-7)

STUDY TYPE: Nontarget Insect Testing (885.4340)

MRID 47444909

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-040

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: OCT 28 2008

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: Anthony Q Armstrong
Date: OCT 28 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: OCT 28 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: OCT 28 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

Primary Reviewer: Eric B. Lewis, Oak Ridge National Laboratory
EPA Reviewer: Shannon Borges, Zigfridas Vaituzis, OPP/BPPD

STUDY TYPE:	Nontarget Insect Testing (885.4340)
MRID NO:	47444909
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:	MSL0021036
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:	Evaluation of Potential for Interaction Between the Bacillus thuringiensis Proteins Cry3Bb1, Cry34Ab1, and Cry35Ab1
AUTHORS:	McRae, T.C.
STUDY COMPLETED:	January 18, 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. According to the study authors, insect handling and details of diet preparation were not documented in the raw data.
STUDY SUMMARY:	A six-day bioassay was conducted to assess the potential for interactive effects between Cry3Bb1 and Cry34Ab1/Cry35Ab1 proteins when fed at nominal concentrations of 0.137 to 100 µg/cm^2 in the diet to neonate southern corn rootworm (*Diabrotica undecimpunctata howardi*) larvae. Diet treated with sterile water served as the untreated control. Model-predicted LC_{50} (mortality) and GI_{50} (growth inhibition) values for larvae fed Cry3Bb1 + Cry34/35Ab1 were not significantly different from the observed LC_{50} and GI_{50} values, indicating there was no statistically significant interaction (synergism or antagonism) between Cry3Bb1 and the Cry34Ab1 and Cry35Ab1 proteins in this study.
CLASSIFICATION:	**Acceptable**

Test Material

Purified *E. coli*-produced Cry3Bb1, stored in 50 mM sodium carbonate/bicarbonate, 1-mM EDTA buffer, pH~10.1; purity 87%, purity-corrected concentration of 4.1 mg/mL; Lot No. 20-100025; supplied by the study sponsor.

Lyophilized Cry34Ab1 protein powder, purity 69.9% (protein to powder mass ratio); Lot No. TSN104463; supplied by Dow AgroSciences and subsequently suspended in 10-mM potassium phosphate buffer, pH~7.5; purity-corrected concentration of 2.0 mg/mL.

Lyophilized Cry35Ab1 protein powder, purity 40.3% (protein to powder mass ratio); Lot No. TSN104462; supplied by Dow AgroSciences and subsequently suspended in 10-mM potassium phosphate buffer, pH~7.5; purity-corrected concentration of 2.0 mg/mL.

The Cry34Ab1 and Cry35Ab1 suspensions were combined in a ratio of 9:1 (w/w) for the bioassays.

Buffers of the same composition and pH but without protein were used as control substances.

The test and control substances were stored at approximately 4°C during the study.

Test Methods

The study was conducted to assess the potential for interactive effects between Cry3Bb1 and Cry34Ab1/Cry35Ab1 proteins. The test organisms were neonate (<24 hours old) southern corn rootworm (SCRW, *Diabrotica undecimpunctata howardi*) hatched from eggs supplied by Crop Characteristics, Inc., Farmington, MN. Each bioassay contained seven concentrations each of Cry3Bb1 and Cry34Ab1/Cry35Ab1 prepared using standard serial dilution techniques to achieve nominal concentrations of 0.137 to 100 µg/cm^2 in diet. The concentrations used were based on results of rangefinding bioassays conducted prior to the study. The Cry3Bb1 and Cry34Ab1/Cry35Ab1 dilution series were used to prepare a third dilution series in which each Cry3Bb1 and Cry34/35Ab1 dilution were combined 1:1 (w/w). Each bioassay also included one treatment of each buffer diluted to the same level as the highest concentration of its corresponding protein, as well as a third buffer treatment consisting of a mixture of the two buffers prepared in the same manner as the Cry3Bb1 + Cry34/35Ab1 mixture. Lastly, each bioassay included two treatments of sterile water to serve as untreated controls.

SCRW insect diet (modified from Marrone, et al., 1985) was dispensed (200 µL/well) into 96-well assay plates and allowed to solidify. The appropriate test or control substance (25 µL/well) was added to the surface of the diet and a randomly chosen larva was placed in each well. The assay plates were then covered with mylar, which was pierced over each well to provide ventilation. The plates were incubated in darkness at 27°C and 60% relative humidity for 6 days, after which the number of live and dead larvae were determined. Live larvae were pooled by treatment and weighed.

Percent mortality and growth inhibition were calculated. Growth inhibition was based on reduction in live weight, which incorporates mortality and yields a measure of efficacy that is proportional to the biomass of live insects (Herman et al., 2002). Bioassay data sets were accepted as valid if untreated control mortality did not exceed 20% and at least three concentrations produced mortality and growth inhibition responses bracketing 50%. Data from valid replicates were pooled

for further analysis. LC_{50} and GI_{50} values were estimated from the mortality data using SAS v. 9.1.3. Using the observed values, predicted LC_{50} and GI_{50} values for the combination of all three proteins were determined based on the simple similar action model (Finney 1971, Tabashnik 1992). If the predicted values fell within the observed 95% confidence limits for the estimated values for LC_{50} and GI_{50}, then it was concluded that no interaction occurred between the Cry3Bb1 and Cry34/35Ab1 proteins.

Results Summary

Results are summarized in Table 1. Two of the five replicates were rejected because the percent growth inhibition did not bracket a 50% response in one of the three treatments. Mortality in the untreated control averaged 11% in the accepted replicates, with corrected (for the untreated control) mortality and growth inhibition of 1% or less in the three buffer controls. The predicted LC_{50} and GI_{50} values for Cry3Bb1 + Cry34/35Ab1 were not significantly different from the observed LC_{50} and GI_{50} values, indicating there is no evidence of interaction (synergism or antagonism) between Cry3Bb1 and the Cry34Ab1 and Cry35Ab1 proteins.

TABLE 1. Observed vs. expected values of protein treatments against SCRW

Treatment	LC_{50} (µg of toxin/cm^2 of diet)					
	Observed			Expected[b]	Interaction type	% Difference from expected
	Mean	Lower[a]	Upper[a]			
Cry3Bb1	68.74	52.46	95.17	--	--	
Cry34/35Ab1	58.55	36.20	98.16	--	--	
Cry3Bb1 + Cry34/35Ab1	65.37	53.51	79.13	63.24	Additive	-3
Treatment	**GI_{50} (µg of toxin/cm^2 of diet)**					
	Observed			Expected[b]	Interaction type	% Difference from expected
	Mean	Lower[a]	Upper[a]			
Cry3Bb1	31.23	18.19	44.28	--	--	
Cry34/35Ab1	4.01	2.48	5.53	--	--	
Cry3Bb1 + Cry34/35Ab1	10.90	6.95	14.85	7.10	Additive	-35

Data from p. 16, MRID 47444909

[a]95% confidence interval

[b]Calculated by $[(0.5/\text{mean Cry3Bb1}) + (0.5 \text{ mean Cry34/35Ab1})]^{-1}$

Study Authors' Conclusions

The study authors concluded that there is no evidence of interaction (synergism or antagonism) between Cry3Bb1 and the Cry34Ab1 and Cry35Ab1 proteins.

EPA Reviewer's Conclusion

The expected values for the LC_{50} and GI_{50} fall within the 95% confidence limits of the values estimated from the toxicity tests. Based on these results there is no evidence of interaction.

23

References

Finney, D.J. 1971. Probit Analysis. Cambridge University Press, London.

Herman, R.A., P.N. Scherer, D.L. Young, et al. 2002. Binary Insecticidal Crystal Protein from *Bacillus thuringiensis*, Strain PS149B1: Effects of Individual Protein Components and Mixtures in Laboratory Bioassays. Journal of Economic Entomology 95(3):635-639.

Marrone, P.G., F.D. Ferri, T.R. Mosley, et al. 1985. Improvements in Laboratory Rearing of the Southern Corn Rootworm, *Diabrotica undecimpuncta howardi* Barber (Coleoptera: Chrysomelidae), on an Artificial Diet and Corn. Journal of Economic Entomology 78(1):290-293.

Tabashnik, B. E. 1992. Evaluation of synergism among *Bacillus thuringiensis* toxins. Applied and Environmental Microbiology 58(10):3343-3346.

DATA EVALUATION RECORD

BACILLUS THURINGIENSIS CRY1A.105 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR PV-ZM IR245) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY2AB2 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR PV-ZM IR245) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS VAR. AIZAWAI CRY1F (SYNPRO) AND THE GENETIC MATERIAL (FROM THE INSERT OF PLASMID PGMA2810) NECESSARY FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY3BB1 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR ZMI R39) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY34AB1 AND CRY35AB1 PROTEINS AND THE GENETIC MATERIAL NECESSARY FOR THEIR PRODUCTION IN CORN
(MON 89034 X TC1507 X MON 88017 X DAS-59122-7)

STUDY TYPE: Product Performance (810.3000)

MRID 47444910

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-040

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: OCT 28 2008

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature: Anthony Q. Armstrong
Date: OCT 28 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: OCT 28 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: OCT 28 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

Primary Reviewer: Eric B. Lewis, Oak Ridge National Laboratory

EPA Reviewer: Shannon Borges, Zigfridas Vaituzis, OPP/BPPD

STUDY TYPE:	Nontarget Insect Testing (885.4340)
MRID NO:	47444910
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:	MSL0021104
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:	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
AUTHORS:	Levine, S.L
STUDY COMPLETED:	February 28, 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.
STUDY SUMMARY:	A laboratory assay was conducted to evaluate 1) the potential for interactions among the lepidopteran-active proteins (Cry1A.105, Cry2Ab2, and Cry1F) and 2) the potential for interactions between the lepidopteran-active proteins and the coleopteran-active proteins (Cry3Bb1 and Cry34/35Ab1) expressed by the combined trait corn event MON 89034 x TC1507 x MON 88017 x DAS-59122-7. To assess interaction among the lepidopteran-active proteins, European corn borer (ECB, *Ostrinia nubilalis*) larvae were exposed to a series of lyophilized leaf tissue concentrations of either MON 89034 (containing Cry1A.105 and Cry2Ab2), TC1507 (containing Cry1F), or MON 89034 x TC1507 x NK603 (containing Cry1A.105, Cry2Ab2, and Cry1F) in diet incorporation assays. Concurrently, ECB larvae were exposed to lyophilized leaf tissue concentrations of either MON 89034 x TC1507 x NK603 or MON 89034 x TC1507 x MON 88017 x DAS-59122-7 to test for interaction between the lepidopteran-active and coleopteran-active Cry proteins. Control treatments included a diet-only assay control, lyophilized conventional

26

corn tissue, lyophilized MON 88017 tissue, and lyophilized DAS-59122-7 tissue. A concentration addition model was used to compare the observed GI_{50} value (50% growth inhibition, based on mean ECB body weight) for MON 89034 x TC1507 x NK603 with the predicted GI_{50} value for MON 89034 x TC1507 X NK603, and the observed GI_{50} values for MON 89034 x TC1507 x NK603 and MON 89034 x TC1507 x MON 88017 x DAS-59122-7. The mean weight of ECB larvae decreased with increasing tissue concentration of each test corn product. There was no significant difference between the estimated and predicted GI_{50} values for MON 89034 x TC1507 x NK603, indicating there was no interaction between the combination of the Cry1A.105 and Cry2Ab2 proteins and the Cry1F protein. The GI_{50} values for MON 89034 x TC1507 x NK603 and MON 89034 x TC1507 x MON 88017 x DAS-59122-7 were not significantly different, indicating that the Cry3Bb1 and Cry34/35Ab1 proteins do not interact with the combined activity of the Cry1A.105, Cry2Ab2, and Cry1F proteins. These results indicate that there is no interaction among the *Bt* proteins expressed in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn.

CLASSIFICATION: **Acceptable**

Product Description

MON 89034 x TC1507 x MON 88017 x DAS-59122-7 is a combined trait corn that confers insect resistance and tolerance to glyphosate and glufosinate herbicides. MON 89034 produces Cry2Ab2 and Cry1A.105 to protect against feeding damage by European corn borer and other lepidopteran pests. TC1507 produces Cry1F to control European corn borer larvae and other lepidopteran pests, as well as the phosphinothricin acetyl transferase (PAT) protein to confer tolerance to glufosinate-ammonium. MON 88017 produces Cry3Bb1 protein to protect against corn rootworm larvae, as well as 5-enolpyruvylshikimate-3-phosphate synthase protein to confer tolerance to glyphosate. DAS-59122-7 produces Cry34Ab1 and Cry35Ab1 proteins to protect against coleopteran pests, as well as the PAT protein to confer tolerance to glufosinate-ammonium.

Test and Control Materials

The test and control materials were lyophilized leaf tissue from plants in the V6 vegetative growth stage. Leaf tissue was produced under Monsanto production plan 07-01-52-07 and stored at -80°C from the time of collection until lyophilization in a VirTis 24X48 GPFD Freeze Dryer (VirTis Co., Gardiner, NY). The lyophilized tissue was analyzed for moisture content.

The test materials were:

MON 89034 (starting seed lot no. GLP-0604-17104-S)

TC1507 (starting seed lot no. GLP-0604-17103-S)
MON 89034 x TC1507 x NK603* (starting seed lot no. GLP-0604-17107-S)
MON 89034 x TC1507 x MON 88017 x DAS-59122-7 (starting seed lot no. GLP-0604-17108-S).

*NK603 expresses the CP4 EPSPS from *Agrobacterium* sp. Strain CP4, which confers tolerance to glyphosate

The control materials were:

MON 88017 (starting seed lot no. GLP-0604-17100-S)
DAS-59122-7 (starting seed lot no. GLP-0604-17101-S)
XE6001 conventional corn standard (starting seed lot no. GLP-0604-17109-S).

All the test and control materials were in the XE6001 hybrid genetic background. A diet-assay only control was also included.

The presence or absence of MON 89034, TC1507, NK603, MON 88017, and DAS-59122-7 in the test and control substances were verified by event-specific polymerase chain reaction analyses conducted by the registrant's Product Characterization Center. Copies of the certificates of analysis for the test and control substance starting seed are included in the registrant's study file.

Test Methods

The purpose of the test was to evaluate the potential for interactions among the proteins produced by MON 89034 x TC1507 x MON 88017 x DAS-59122-7. This was accomplished by concurrently assessing 1) the potential for interactions among the lepidopteran-active proteins (a combination of Cry1A.105 and Cry2Ab2 proteins with the Cry1F protein) and 2) the potential for interactions between the lepidopteran-active proteins and the coleopteran-active proteins Cry3Bb1 and Cry34/35Ab1.

MON 89034 x TC1507 x NK603 was used to assess the potential for interaction between the lepidopteran proteins because it expresses the Cry1A.105, Cry2Ab2, and Cry1F proteins, but not the Cry3Bb1 and Cry34/35Ab1 proteins. To perform the assessment, European corn borer (ECB, *Ostrinia nubilalis*) larvae were exposed to a series of lyophilized leaf tissue concentrations of either MON 89034, TC1507, or MON 89034 x TC1507 x NK603 in diet incorporation assays run in parallel to estimate the GI_{50} values (50% growth inhibition) for comparison to predicted values based on the GI_{50} for each individual component (see below).

To assess whether combined Cry1A.105, Cry2Ab2, and Cry1F activity is altered by the presence of Cry3Bb1 and Cry34/35Ab1, ECB were exposed to a series of lyophilized leaf tissue concentrations of either MON 89034 x TC1507 x NK603 or MON 89034 x TC1507 x MON 88017 x DAS-59122-7 in diet incorporation assays run in parallel. The estimated GI_{50} values from MON 89034 x TC1507 x NK603 and MON 89034 x TC1507 x MON 88017 x DAS-59122-7 were then compared statistically.

Control treatments included a diet-only assay control, lyophilized conventional corn tissue, lyophilized MON 88017 tissue, and lyophilized DAS-59122-7 tissue.

28

The diet-incorporation bioassays were conducted according to Monsanto SOP BR-ME-0044. A single tissue-diet concentration was tested for MON 88017, DAS-59122-7, and the XE6001 control, and was equivalent to the amount of tissue in the highest concentration tested for MON 89034 x TC1507 x NK603 and MON 89034 x TC1507 x MON 88017 x DAS-59122-7. The test concentrations for MON 89034, MON 89034 x TC1507 x NK603, and MON 89034 x TC1507 x MON 88017 x DAS-59122-7 ranged from 0.16 to 0.256 mg tissue/mL of diet, with a two-fold dilution factor between each concentration level. The test concentrations for TC1507 ranged from 0.40 to 6.4 mg tissue/mL of diet, with a two-fold dilution factor between each concentration level. Dosing solutions for each concentration level and each protein were prepared independently. All dietary concentrations were based on results of rangefinding assays.

Treatments were prepared by mixing 5 mL of purified water with finely-ground lyophilized leaf tissue and adding 20 mL of warm (52-54°C) agar-based multi-species diet (Southland Products) to a final volume of 25 mL. The diet was vortex-mixed until homogeneous. The diet-only assay controls contained 5 mL of purified water and 20 mL of diet. The diets were then placed into individual wells of 128-well bioassay trays (0.5 mL/well) and allowed to solidify.

ECB eggs were obtained from Monsanto's Waterman, IL facility and handled according to Monsanto SOP BR-ME-0989. One newly-hatched larva (≤24 hours after first observed hatching) was added to each well and the trays were covered with a ventilated adhesive cover. Each replicate contained 24 larvae. One replicate was maintained for each test concentration and three replicates for each control treatment. The trays were incubated for seven days at 27°C and ambient humidity with a 14:10 light:dark photoperiod. At test end, the number of survivors in each treatment and the combined weight of the survivors in each treatment were determined. Assays with the test and control substances were run concurrently using the same batch of insects.

Mean body weights among the control treatments were compared using a mixed regression model in SAS. A joint logistic model analysis was run under PROC NLMIXED in SAS to 1) model the concentration responses and estimate GI_{50} values for MON 89034, TC1507, MON 89034 x TC1507 x NK603, and MON 89034 x TC1507 x MON 88017 x DAS-59122-7, and 2) to statistically compare the GI_{50} values for MON 89034 x TC1507 x NK603 and MON 89034 x TC1507 x MON 88017 x DAS-59122-7.

The potential for interaction between the Cry1A.105 + Cry2Ab2 and Cry1F proteins was determined by comparing the estimated and predicted GI_{50} values for MON 89034 x TC1507 x NK603. The predicted GI_{50} value for MON 89034 x TC1507 x NK603 is a function of the GI_{50} values for MON 89034 and TC1507 and was based on a concentration addition model (Finney, 1971; Tabashnik, 1992). Statistical significance was determined at the 95% confidence level.

Results Summary

The mean weight of ECB larvae decreased with increasing tissue concentration of MON 89034, TC1507, MON 89034 x TC1507 x NK603 and MON 89034 x TC1507 x MON 88017 x DAS-59122-7 (Table 1). Slopes of GI_{50} curves for all groups were visibly comparable (see Fig. 2, page 23 of MRID 47444910), which is consistent with similar modes of action.

TABLE 1. Mean ECB weight by treatment and tissue concentration level in diet		
Treatment	**Concentration (mg tissue/mL of diet)**	**Mean weight (mg)**
Diet only	0.256	16.88 ± 2.23
XE6001	0.256	17.48 ± 2.94
MON 88017	0.256	17.85 ± 2.17
DAS-59122-7	0.256	17.00 ± 1.87
MON 89034	0.016	17.75 ± 4.93
	0.032	10.05 ± 0.81
	0.064	7.11 ± 0.73
	0.128	3.81 ± 0.08
	0.256	1.38 ± 0.24
TC1507	0.400	14.42 ± 2.28
	0.800	10.33 ± 1.70
	1.600	6.86 ± 0.67
	3.200	4.07 ± 0.27
	6.400	2.59 ± 0.21
MON 89034 x TC1507 x NK603	0.016	13.97 ± 3.35
	0.032	11.25 ± 3.01
	0.064	7.08 ± 1.16
	0.128	3.43 ± 0.31
	0.256	1.10 ± 0.16
MON 89034 x TC1507 x MON 88017 x DAS-59122-7	0.016	15.75 ± 3.82
	0.032	11.17 ± 2.00
	0.064	7.18 ± 1.02
	0.128	2.98 ± 0.52
	0.256	1.06 ± 0.08

Data from p. 30, MRID 47444910

Table 2 lists the parameter estimates from the logistic model used to estimate the concentration-response for MON 89034, TC1507, MON 89034 x TC1507 x NK603, and MON 89034 x TC1507 x MON 88017 x DAS-59122-7, as well as the predicted GI_{50} value for MON 89034 x TC1507 x NK603. Concentrations associated with mean weights were similar for all treatments except for that of TC1507 (expressed as GI_{502} in the table) indicating that Cry1F is less potent to ECB. This result is not unexpected.

TABLE 2. Parameter estimation		
Parameter	**Estimate**	**Standard error**
GI_{501} (mg tissue/mL diet)	0.0523	0.0061
GI_{502} (mg tissue/mL diet)	1.7294	0.2176
GI_{503} (mg tissue/mL diet)	0.0478	0.0054
GI_{504} (mg tissue/mL diet)	0.0458	0.0051
B	1.5180	0.0863

Data from p. 32, MRID 47444910

GI_{50}: Concentration for 50% growth inhibition in the joint analysis for MON 89034 (with GI_{501}) and TC1507 (with GI_{502}) and MON 89034 x TC1507 X NK603 (with GI_{503}) and MON 89034 x TC1507 x MON88017 x DAS-59122-7 (with GI_{504}) and diet only as the common control for zero concentration

B: rate parameter of the weight change with concentration

Table 3 provides the results of the significance testing of the difference between the predicted and estimated values. There was no significant difference between the estimated and predicted GI_{50} values for MON 89034 x TC1507 x NK603 (values for these were 0.0478 and 0.0508, respectively, p = 0.53), indicating that there was no interaction between the combination of the Cry1A.105 and Cry2Ab2 proteins and the Cry1F protein. The GI_{50} values for MON 89034 x TC1507 x NK603 and MON 89034 x TC1507 x MON 88017 x DAS-59122-7 were not significantly different, indicating that the Cry3Bb1 and Cry34/35Ab1 proteins do not interact with the combined activity of the Cry1A.105, Cry2Ab2, and Cry1F proteins.

TABLE 3. Significance testing for difference between the estimated GI_{50} value for MON 89034 x TC1507 x NK603 and the prediction from MON 89034 and TC1507, and significance testing of $GI_{503} = GI_{504}$ for MON 89034 x TC1507 x NK603 and MON 89034 x TC1507 x MON 88017 x DAS-59122-7

Parameter/hypothesis	Estimate	Std error	DF	t Value	p Value
Predicted GI_{503}	0.0508	0.0059			
H_0: Estimated GI_{503}- Predicted $GI_{503} = 0$	-0.0029	0.0034	62	-0.88	0.3841
H_0: $GI_{503} - GI_{504} = 0$	0.0020	0.0032	62	0.63	0.5286

Data from p. 32, MRID 47444910

Study Authors' Conclusions

The study authors concluded that there was no interaction among the *Bt* proteins expressed in MON 89034 x TC1507 x MON 88017 x DAS-59122-7.

EPA Reviewer's Conclusion

Based on the data presented and the models used to estimate values for comparison, the combined activity of the Cry proteins, Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, and Cry34/35Ab1 is not synergistic or antagonistic.

References

Finney, D.J. 1971. Probit Analysis. Cambridge University Press, London. 333 pp.

Tabashnik, B.E. 1992. Evaluation of a Synergism Among *Bacillus thuringiensis* Toxins. Applied and Environmental Microbiology 58:3343-3346.

DATA EVALUATION RECORD

BACILLUS THURINGIENSIS CRY1A.105 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR PV-ZM IR245) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY2AB2 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR PV-ZM IR245) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS VAR. AIZAWAI CRY1F (SYNPRO) AND THE GENETIC MATERIAL (FROM THE INSERT OF PLASMID PGMA2810) NECESSARY FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY3BB1 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR ZM1 R39) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY34AB1 AND CRY35AB1 PROTEINS AND THE GENETIC MATERIAL NECESSARY FOR THEIR PRODUCTION IN CORN
(MON 89034 X TC1507 X MON 88017 X DAS-59122-7)

STUDY TYPE: Endangered Species Impact Assessment (Nonguideline)

MRID 47444912

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-040

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: OCT 28 2009

Secondary Reviewers:
Anthony Q. Armstrong, M.S.

Signature:
Date: OCT 28 2009

Robert H. Ross, M.S., Group Leader

Signature:
Date: OCT 28 2009

Quality Assurance:
Lee Ann Wilson, M.A.

Signature:
Date: OCT 28 2009

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

Primary Reviewer: Eric B. Lewis, Oak Ridge National Laboratory
EPA Reviewer: Shannon Borges, Zigfridas Vaituzis, OPP/BPPD

STUDY TYPE:	Endangered Species Impact Assessment (Non-guideline)
MRID NO:	47444912
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:	MSL0021268
SPONSOR:	Monsanto Company, 800 North Lindbergh Blvd., St. Louis, MO 63167
TESTING FACILITY:	N/A
TITLE OF REPORT:	Endangered Species Impact Assessment for the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7
AUTHORS:	Huesing, J.E. and S.L. Levine
STUDY COMPLETED:	April 16, 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:	An impact assessment for threatened and endangered species was conducted for the combined trait corn product MON 89034 x TC1507 x MON 88017 x DAS-59122-7, which produces the lepidopteran–active *Bacillus thuringiensis* (*Bt*) Cry1A.105, Cry2Ab2, and Cry1F proteins and the coleopteran-active *Bt* Cry3Bb1, Cry34Ab1, and Cry35Ab1 proteins. Due to the specificity of Cry1A.105, Cry2Ab2, and Cry1F, species outside the insect order Lepidoptera should not be affected. Within Lepidoptera, the only potential concern is for the Karner blue butterfly (*Lycaeides melissa samuelis*); however, habitat characteristics and feeding biology of the Karner blue essentially eliminate any potential exposure to MON 89034 x TC1507 x MON 88017 x DAS-59122-7 pollen. Due to the specificity of Cry3Bb1 and Cry34/35Ab1, species outside the insect order Coleoptera should not be affected. Within Coleoptera, the only species with a range

	overlapping corn production areas is the American burying beetle (*Nicrophorus americanus*); however, this species feeds only on carrion and there is little chance of exposure. While several threatened and endangered coleopteran species occur in aquatic habitats, an analysis of the potential impact from aquatic exposure to Cry3Bb1 and Cry34/35Ab1 via pollen drift indicates that these species will not be adversely affected.
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, 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.

In previous ecological effects assessments for MON 89034, TC1507, MON 88017, and DAS-59122-7, EPA concluded that no adverse effects to listed threatened or endangered animal species or their critical habitat were expected from cultivation, and no threatened or endangered plant species would be affected by outcrossing to wild relatives or by competition with MON 89034, TC1507, MON 88017, or DAS-59122-7 (US EPA, 2005a, 2005b, 2007, 2008). The activity of the *Bt* Cry proteins depends on their binding to specific receptors present in the insect mid-gut (OECD, 2007; Pigott and Ellar, 2007; Lambert et al., 1996; Van Rie et al., 1989, 1990; Hofmann et al., 1988a, 1988b; Wolfersberger et al., 1986). These specific receptors are not present in non-target birds and wild mammals; therefore the Cry proteins are not expected to adversely affect these organisms (OECD, 2007; Shimada et al., 2006; Mendelsohn et al., 2003; Lambert et al., 1996; Van Rie et al., 1989, 1990; Hofmann et al., 1988a, 1988b; Wolfersberger et al, 1986; Sacchi et al., 1986).

Insecticidal activity spectrum studies and non-target organism studies with vertebrates and invertebrates have been submitted to EPA for the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, and Cry34/35Ab1 proteins (Head et al., 2001; MacRae et al., 2006a, 2006b; Herman and Korjagin, 1999; Herman et al., 2000). Bioassays conducted with the lepidopteran-active Cry1A.105, Cry2Ab2, and Cry1F proteins and with the coleopteran-active Cry3Bb1 and Cry34/35Ab1 proteins demonstrated that, at field exposure concentrations, activity is restricted to lepidopteran and coleopteran insects, respectively. Given the specificity for the *Bt* Cry proteins produced by MON 89034 x TC1507 x MON 88017 x DAS-59122-7, the endangered species assessment focused on the orders Lepidoptera and Coleoptera.

Threatened and endangered lepidopteran and coleopteran species in the US have very restricted habitat ranges and their larvae typically feed on specific host plants, none of which include corn or its sexually-compatible relatives. Furthermore, lepidopteran insects do not occur in aquatic environments. County-level distributions of threatened or endangered lepidopteran species indicate that the potential concern for range overlap with corn production is restricted to the

Karner blue butterfly (*Lycaeides melissa samuelis*). County-level distributions of threatened or endangered coleopteran species indicate that the potential concern for range overlap with corn production is restricted to the American burying beetle (*Nicrophorus americanus*). Several threatened or endangered coleopteran species are aquatic species, and as such are not expected to occur in or near corn fields; nevertheless aquatic coleopteran species were included in the risk assessment.

Potential Impact to the Karner Blue Butterfly

The potential for impact to the Karner blue butterfly from exposure to *Bt* corn has previously been addressed (Mendelsohn et al., 2003; Peterson et al., 2006). Karner blues are found in parts of Wisconsin, Michigan, Minnesota, Indiana, New Hampshire, and New York (Peterson et al., 2006; US EPA, 2001: USFWS, 2003). There are two generations of Karner blue each year. First generation larvae hatch in mid-April, well before corn pollen shed in these areas. Second generation larvae feed through mid-July. Temporal desynchrony between the presence of Karner blue larvae feeding and maize pollen shed limits the potential exposure (Mendelsohn et al., 2003).

Wild lupine is the only known host for Karner blue larvae. Wild lupine grows on dry, sandy soils in pine barrens, oak savannahs, and disturbed habitats (USFWS, 2003: Peterson et al., 2006). Wild lupine does not occur in corn fields. Data from Peterson et al. (2006) indicate that <10% of all lupine sites are within 100 m of a corn field, and 80% are >500 m from a corn field.

For Karner blue butterfly larvae to be exposed to pollen from MON 89034 x TC1507 x MON 88017 x DAS-59122-7, lupine plants must be in or around corn fields, and pollen shed must occur into the lupine at the time when larvae are feeding. In the corn zones where Karner blues occur, corn plants typically shed pollen after mid-to-late July or early August. Degree-day effects that might result in earlier or later pollen shed would also tend to correspondingly affect the rate of Karner blue development, and not increase the likelihood of exposure. Peterson et al. (2006) showed that there are only two Wisconsin counties where there is potential temporal overlap between Karner blue larvae and corn pollen shed. However, there is no evidence that the larvae are exposed to harmful levels of corn pollen in these two locations.

Most Sensitive Lepidopteran Species Risk Analysis

In independent dietary toxicity tests using purified Cry1A.105, Cry2Ab2, and Cry1F proteins (Levine, 2006: MacRae et al., 2006a, 2006b, 2006c; Wolt et al., 2005) the European corn borer (ECB, *Ostrinia nubilalis*) was the most sensitive species. The lowest LC_{50} values for the CryA.105, Cry2Ab2, and Cry1F proteins in those studies were 0.43, 1.5, and 0.58 µg/mL of diet, respectively. In a study using ECB to assess the potential for interaction among the *Bt* Cry proteins produced by MON 89034 x TC1507 x MON 88017 x DAS-59122-7, no interaction was found (Levine et al., 2008). The biological activity of the lepidopteran-specific proteins was found to be additive in that study.

For the most sensitive lepidopteran species assessment, the biological activity of CryA.105, Cry2Ab2, and Cry1F were conservatively considered to be equipotent against ECB. The levels of CryA.105, Cry2Ab2, and Cry1F proteins in pollen from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 are 14.2, 1.5, and 18.6 µg/g fresh weight, respectively, with a combined level of 34

36

µg/g fresh weight. Based on the 12-day LC_{50} for ECB larvae and the 95th percentile exposure level in pollen-covered food, the safety factors (margins of safety) were calculated for exposure to corn pollen drift in terrestrial environments (Table 1). A ≥10-fold safety factor is achieved at distances ≥1.74 m from the edge of the corn field. As noted above, less than 10% of all lupine sites are within 100 m of a corn field, and approximately 80% of all lupine sites are more than 500 m from a corn field. It is therefore highly unlikely that lupine occurs within two meters of a corn field where the highest pollen exposures occur. Furthermore, in the two Wisconsin counties where a potential temporal overlap exists between Karner blue larvae and corn pollen, there is presently no evidence that the larvae are exposed to harmful levels of corn pollen at these locations.

TABLE 1. Estimated exposure, hazard, and calculated safety factors for the combined CryA.105, Cry2Ab2, and Cry1F proteins in pollen from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 deposited on host plant leaf surfaces[1]

Distance from edge of field (m)	Pollen deposition on leaf surface (grains/cm²)	Dietary exposure for combined Cry proteins (µg/g diet)[2]	LC50 for the most sensitive species (µg/g diet)[3]	Safety factor[4]
1.74	101	0.043	0.43	10
2	75	0.032[5]	0.43	13
4-5	25	0.010	0.43	40

Data from p. 29, MRID 47444912, modified with corrections.

[1]The model used for these calculations is taken in part from Pleasants et al. (2001) and Dively et al. (2004). The model is not presented; however, Pleasants et al. (2001) report 95% upper confidence limits for pollen deposited at 1, 2, and 4-5 meters from the cornfield edge to be 200, 75, and 25 pollen grains/cm².

[2]The combined CryA.105, Cry2Ab2, and Cry1F exposure concentrations on host plant leaf surfaces. Dietary exposure was calculated with the equation ([(a/b) x c]/d) where:

a = no. of pollen grains deposited per cm² on milkweed leaves (surrogate food substrate) (Pleasants et al., 2001).

b = no. of pollen grains per g of fresh weight in corn pollen (~4 x 10^6 grains according to Miller, 1985)

c = the combined Cry protein concentration of 34 µg/g fresh weight of corn pollen: 14.2 µg/g Cry1A.105 + 18.6 µg/g Cry1F + 1.5 µg/g Cry2Ab2 (Stillwell and Silvanovich, 2007; Phillips, 2008). Individual Cry protein levels represent the upper 95th percentile of the expression values. Cry1F protein expression values were converted from dry weight to wet weight, considering that dry weight is 65% of fresh weight.

d = fresh weight (g) of the food plants per cm² (~0.02 g/cm² of fresh milkweed leaves according to information in Hellmich et al., 2001).

[3]It was assumed that each mL of diet weighs approximately one gram, allowing conversion from µg/mL to µg/g of diet.

[4]The safety factor was calculated as the quotient of the 12-day ECB LC_{50} value (MacRae et al., 2006) divided by the combined exposure concentration for CryA.105, Cry2Ab2, and Cry1F, which were assumed to have equivalent potency against ECB.

[5]The value listed here in the original report was 0.029 µg/g diet. Based on the model described the value should be 0.032, which agrees with the rest of the value of 13 for the safety factor at 2 meters from the corn field edge.

Endangered Coleopteran Species – Risk Characterization

Cry3Bb1 and Cry34/35Ab1 are specific to coleopteran species. According to the author, there are no reports of threatened or endangered coleopteran species feeding directly on corn plants. Of the

37

16 threatened or endangered coleopteran species, the only one with a potential range overlap with corn production counties is the American burying beetle (*Nicrophorus americanus*). Both adults and larvae of the American burying beetle feed exclusively on carrion, and if they did occur in proximity to MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn fields, there would be little chance of exposure to Cry3Bb1 and Cry34/35Ab1 proteins due to their feeding habits.

Aquatic Insects – Risk Characterization

Lepidopteran insects do not occur in aquatic environments (Triplehorn and Johnson, 2007). Insects in the order Trichoptera are the closest relatives to the Lepidoptera and do occur as immatures in aquatic habitats. While Rosi-Marshall et al. (2007) observed negative effects of *Bt* Cry proteins on certain Trichopteran species, their methodology and conclusions have not been supported by other investigators and are inconsistent with their earlier conclusions (Beachy et al., 2008; Parrott, 2008; Rosi-Marshall et al., 2008). Jensen et al. (2007) found no negative effects of Cry1Ab or Cry3Bb1 on aquatic Trichoptera.

There are currently several threatened and endangered coleopteran species that occur in aquatic environments, but potential exposure to corn tissues is minimal (EPA, 2005b). Runoff of Cry3Bb1 and Cry34/35Ab1 proteins into adjacent water bodies, either as free protein of as protein sequestered in plant debris, is expected to be extremely low. When lyophilized corn tissues containing Cry3Bb1 or Cry34/35Ab1 were added to standard agricultural soils, the dissipation half-lives were approximately three days (US EPA, 2007, 2005b; Herman et al., 2000). Therefore, pollen drift was used as the predictor for potential risk to endangered aquatic coleopterans.

Expression analysis of pollen from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 showed that the 95th percentiles for Cry34Ab1 and Cry3Bb1 are 72 and 18 µg/g fresh weight, respectively. The level of Cry35Ab1 was below the limit of detection in the majority of the samples (Phillips, 2008). Therefore, only the levels of Cry3Bb1 and Cry34Ab1 in pollen were summed to determine the combined concentration. Cry3Bb1 and Cry34/35Ab1 protein activity was found to be additive against southern corn rootworm (*Diabrotica undecimpunctata howardi*) (MacRae, 2008). The analysis assumes 100% pollen deposition to the surface water of a pond adjacent to the corn field. For aquatic species, a 20-fold safety factor based on the LC_{50} for the most sensitive species tested is used to accommodate uncertainty for the particular endangered species.

The combined Cry3Bb1 and Cry34Ab1 protein concentration in the surface water of a one hectare, two meter-deep pond located one meter from the edge of a corn field was calculated to be several orders of magnitude below the toxic level seen in the most sensitive coleopteran species tested for Cry3Bb1 or Cry34/35Ab1 proteins (Table 2). The resulting safety factors were >7000. These results indicate that Cry3Bb1 and Cry34/35Ab1 proteins in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 pollen would have no adverse effect on endangered aquatic beetles.

TABLE 2. Estimated exposure, hazard, and calculated safety factors for the combined Cry3Bb1 and Cry34Ab1 proteins from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 pollen drift to the surface water of a 1-ha pond adjacent to a corn field

Distance from edge of field (m)	Pollen deposition on water surface (grains/cm^2)[a]	Pollen on water surface (grains/ha)[b]	Combined Cry proteins from pollen drift (µg/L)[c]	Combined Cry proteins in the water of a 1-ha pond with a 2-m depth (µg/L)[d]	Most sensitive species LC_{50} (µg/g diet)[e]	Safety factor[f]
1	500	5.00×10^{10}	1,125,000	5.625×10^{-5}	0.41	7289
2	185	1.85×10^{10}	416,250	2.08125×10^{-5}	0.41	19,700
4-5	63	6.30×10^{9}	141,750	7.0875×10^{-6}	0.41	57,848

Data from p. 30, MRID 47444912

[a]The no. of pollen grains deposited on the surface water was estimated based on the assumption that the surface water adjacent to a corn field captured 100% of airborne pollen. Pleasants et al. (2001) reported that wild milkweed leaves intercepted and retained about 40% of airborne pollen based on deposition on sticky microscope slides under similar conditions. When adjusted to the pollen capture rate of 100%, the pollen grains deposited per unit areas of milkweed leaves are equal to the pollen grains captured by the same unit area of surface water.

[b]Total pollen grains in a 1-ha pond surface water was calculated as: no. of pollen grains/cm^2 x 10^8 cm^2/ha.

[c]The combined Cry3Bb1 and Cry34Ab1 protein concentration in the pond was estimated using the 95th percentiles for expression in MON 89034 x TC1507 x MON 88017 x DAS-59122-7 pollen (Stillwell and Silvanovich, 2007; Phillips, 2008). Cry34Ab1 expression values were converted from dry weight to wet weight, considering that dry weight is 65% of fresh weight. The total estimated value was 90 ug protein/g pollen.

[d]The maximum exposure concentration for combined Cry3Bb1 + Cry34Ab1 was estimated based on a total water volume of 2 x 10^7 L. For the exposure assessment, it is conservatively assumed that the concentration of protein in the diet is equal to that in the water.

[e]From a 7-day assay with Colorado potato beetle (*Leptinotarsa decemlineata*) (MacRae et al., 2006c).

[f]Safety factors were calculated as the quotient of the LC_{50} values and the combined Cry3Bb1 and Cry34Ab1 protein concentration in the water.

Study Authors' Conclusions

The study authors concluded that no adverse impacts to threatened and endangered species are anticipated from cultivation of MON 89034 x TC1507 x MON 88017 x DAS-59122-7.

EPA Reviewer's Conclusion

The reviewer agrees with the assessment that the listed lepidopteran and coleopteran species provided in Tables 1 and 2 of the report would be excluded from exposure due to the habitats they occupy and/or restricted feeding habits. A LOCATES search was performed for listed species co-occurring in counties with corn, and there are some discrepancies in the results from LOCATES and the list of threatened and endangered species provided in Tables 1 and 2. The LOCATES search resulted in two additional lepidopteran species and four additional coleopteran species.

The lepidopteran species include the Oregon silverspot butterfly (*Speyeria zerene hippolyta*) and the Saint Francis' satyr butterfly (*Neonympha mitchellii francisci*). The Oregon silverspot butterfly lives along the Pacific coast and occupies early successional salt spray meadows that are influenced by proximity to the ocean. It is limited to coastal grasslands that contain specific host plants. The Saint Francis' satyr butterfly occupies sedge wetlands and open seepage areas in

North Carolina and Virginia, and is not highly mobile. Neither of these species is expected to be found in or around corn fields due to their habitat preferences.

The additional coleopteran species identified by LOCATES includes the Comal Springs dryopid beetle (*Stygoparnus comalensis*), Helotes mold beetle (*Batrisodes venyivi*), *Rhadine exilis*, and *Rhadine infernalis*. The Comal Springs dryopid beetle is limited to outlets of Comal Springs and is believed to be a subterranean obligate. It is not found beyond these outlets. The remaining beetles are known to be subterranean obligates. Therefore, these additional coleopteran species are not expected to be found in or around corn fields.

Based on the information presented for terrestrial and aquatic exposure to MON 89034 x TC1507 x MON 88017 x DAS-59122-7 plant tissue or pollen. Therefore, there will be No Effect (NE), direct or indirect, on endangered and threatened species or their habitat as listed by the United States Fish and Wildlife Service (USFWS) and the National Marine Fisheries Services (NMFS), including mammals, birds or terrestrial and aquatic plants and invertebrate species. Therefore, no consultation with the USFWS is required under the Endangered Species Act.

References

Beachy, R., N. Fedoroff, R. Goldberg, et al. 2008. The Burden of Proof: A Response to Rosi-Marshall et al. Proc. Natl. Acad. Sci. USA 105(7):E9.

Dively, G., R. Rose, M. Sears, et al. 2004. Effects on Monarch Butterfly Larvae (Lepidoptera: Danaidae) After Continuous Exposure to Cry1Ab Expressing Corn During Anthesis. Environ. Entomol. 33:1116-1125.

Head, G., M. Pleau, S. Sivausupramanian, et al. 2001. Insecticidal Spectrum of Activity for Cry3Bb Protein *in vitro*. Monsanto Internal Report Number C3NTO.

Hellmich, R., B. Siegfried, M. Sears, et al. 2001. Monarch Larvae Sensitivity to *Bacillus thuringiensis*-Purified Proteins and Pollen. Proc. Natl. Acad. Sci. USA 98:11925-11930.

Herman, R.A. and V.A. Korjagin. 1999. Microbial B.t. Cry1F (truncated) Delta-Endotoxin: Maize-Insect-Pest Susceptibility Study. Study 990029, Unpublished Report of Dow AgroSciences LLC. MRID 45020201.

Herman, R.A., R.A. Collins, and D.L. Young. 2000. Degradation of Microbial Binary PS 149B1 Delta-Endotoxin in a Representative Soil from the Mid-Western USA Maize-Growing Region. MRID 45242214. Internal Report 000365 DAS LLC.

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.

Jensen, P., W. Lamp, C. Swan, et al. 2007. Examining the Risk to Non-Target Arthopods from Bt Corn Tissue in Agricultural Streams. National Meeting of the Entomological Society of America, San Diego, CA. December, 2007. Paper 0903.

Lambert, B., L. Buysse, C. Decock, et al. 1996. A *Bacillus thuringiensis* Insecticidal Crystal Protein with a High Activity Against Members of the Family Noctuidae. Appl. Envir. Microbiol. 62(1):80-86.

Levine, S.L. 2006. Characterization of Insecticidal Activity of the Cry1A.105 *Bacillus thuringiensis* Protein Against Five Lepidopteran Pests of Corn in 7-Day Laboratory Bioassays. Monsanto Technical Report Number 06-RA-39-08.

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.

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

MacRae, T., C. Brown, and L. Levine. 2006a. Spectrum of Insecticidal Activity of *Bacillus thuringiensis* Cry1A.105 Protein. Monsanto Technical Report Number MSL-20230. St. Louis, MO.

MacRae, T., C. Brown, and L. Levine. 2006b. Spectrum of Insecticidal Activity of *Bacillus thuringiensis* Cry2Ab2 Protein. Monsanto Technical Report Number MSL-20229. St. Louis, MO.

MacRae, T., C. Brown, and L. Levine. 2006c. Evaluation of Potential for Interactions Between the *Bacillus thuringiensis* Proteins Cry1A.105, Cry2Ab2, and Cry3Bb1. Monsanto Technical Report Number MSL-20270. St Louis, MO.

Mendelsohn, M., J. Kough, Z. Vaituzis, et al. 2003. Are *Bt* Crops Safe? Nature Biotechnol. 21:1003-1009.

Miller, P. 1985. Maize Pollen: Collection and Enzymology. Chapter 45, pp. 279-282.

OECD. 2007. Consensus Document on Safety Information on Transgenic Plants Expressing *Bacillus thuringiensis*. OECD Environment, Health and Safety Publications Series on Harmonisation of Regulatory Oversight in Biotechnology. ENV/JM/MONO(2007)14.

Parrott, W. 2008. Study of *Bt* Impact on Caddisflies Overstates Its Conclusions: Response to Rosi-Marshall et al. Proc. Natl. Acad. Sci. USA 105(7):E10.

Peterson, R., S. Meyer, A. Wolf, et al. 2006. Genetically Engineered Plants, Endangered Species and Risk: A Temporal and Spatial Exposure Assessment for Karner Blue Butterfly Larvae and Bt Maize Pollen. Risk Analysis 26(3):845-858.

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.

Pigott, E. and D.J. Ellar. 2007. Role of Receptors in *Bacillus thuringiensis* Crystal Toxin Activity. Microbiol. Mol. Biol. Rev. 71:255-281.

Pleasants, J., R. Hellmich, G. Dively et al. 2001. Corn Pollen Deposition on Milkweeds in and Near Cornfields. Proc. Natl. Acad. Sci. USA 98:11919-11924.

Rosi-Marshall, E., J.L Tank, T.V. Royer, et al. 2007. Toxins in Transgenic Crop Byproducts May Affect Headwater Stream Ecosystems. Proc. Natl. Acad. Sci. USA 104(41):16204-16208.

Rosi-Marshall, E., J.L. Tank, T.V. Royer, et al. 2008. Reply to Beach et al. and Parrott: Study Indicates *Bt* Corn May Affect Caddisflies. Proc. Natl. Acad. Sci. USA 105(7):E11.

Sacchi, V.F., P. Parenti, G.M. Hanozet, et al. 1986. *Bacillus thuringiensis* Toxin Inhibits K+-Gradient-Dependent Amino Acid Transport Across the Brush Border Membrane of *Pieris brassicae* Midgut Cells. FEBS Letters 204:213.

Shimada, N., K. Miyamoto, K. Kanda, et al. 2006. *Bacillus thuringiensis* Insecticidal Cry1Ab Toxin Does Not Affect the Membrane Integrity of the Mammalian Intestinal Epithelial Cell: An in vitro Study. In Vitro Cellular & Developmental Biology – Animal 42:45-49.

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.

Triplehorn, C. and N. Johnson. 2007. Borror and Delong's Introduction to the Study of Insects. Brooks/Cole, Belmont, CA.

US EPA. 2005a. Biopesticides Registration Action document: *Bacillus thuringiensis* Cry1F Corn (updated August, 2005). US EPA, Washington, DC.

US EPA. 2005b. Biopesticides Registration Action document: *Bacillus thuringiensis* Cry34/35Ab1 Corn. US Environmental Protection Agency, Washington, DC.

US EPA. 2007. Biopesticides Registration Action document: *Bacillus thuringiensis* Cry3Bb1 Corn. US Environmental Protection Agency, Washington, DC.

42

US EPA. 2008. Biopesticides Registration Action document: *Bacillus thuringiensis* MON 89034 Corn (updated August, 2005). US Environmental Protection Agency, Washington, DC.

US EPA. 2001. Bt Plant-Incorporated Protectants (October 15, 2001). Biopesticides Registration Action Document. US Environmental Protection Agency, Washington, DC.

USFWS. 2003. Final Recovery Plan for the Karner Blue Butterfly (*Lycaeides mlissa samuelis*). US Fish and Wildlife Service, Fort Snelling, MN. 273 pp. http://ecos.fws.gov/docs/recovery_plans/2003/030919.pdf

Van Rie, J. S. Jansens, H. Hofte, et al. 1989. Specificity of *Bacillus thuringiensis* Delta-Endotoxins, Importance of Specific Receptors on the Brush Border Membrane of the Mid-Gut of Target Insects. Eur. J. Biochem. 186:239-247.

Van Rie, J. S. Jansens, H. Hofte, et al. 1990. Receptors on the Brush Border Membrane of the Insect Midgut as Determinants of the Specificity of *Bacillus thuringiensis* Delta-Endotoxins. Appl. Environ. Microbiol. 56:1378-1385.

Wolfersberger, M.G., C. Hofman, and P. Luthy. 1986. In: Bacterial Protein Toxins (P. Falmagne, J.E. Alout, F.J. Fehrenbach, et al., eds.) pp. 237-238. Fischer, New York.

Wolt, J., C. Conlan, and K Majima. 2005. An Ecological Risk Assessment of Cry1F Maize Pollen Impact to Pale Grass Blue Butterfly. Environ. Biosafety Res. 4:243-251.

DATA EVALUATION RECORD

BACILLUS THURINGIENSIS CRY1A.105 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR PV-ZM IR245) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY2AB2 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR PV-ZM IR245) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS VAR. AIZAWAI CRY1F (SYNPRO) AND THE GENETIC MATERIAL (FROM THE INSERT OF PLASMID PGMA2810) NECESSARY FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY3BB1 PROTEIN AND THE GENETIC MATERIAL NECESSARY (VECTOR ZMI R39) FOR ITS PRODUCTION IN CORN
BACILLUS THURINGIENSIS CRY34AB1 AND CRY35AB1 PROTEINS AND THE GENETIC MATERIAL NECESSARY FOR THEIR PRODUCTION IN CORN
(MON 89034 X TC1507 X MON 88017 X DAS-59122-7)

STUDY TYPE: Nontarget Insect Testing (885.4340)

MRID 47444913

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 08-040

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: OCT 28 2008

Secondary Reviewers:
Anthony O. Armstrong, M.S.

Signature: Anthony O. Armstrong
Date: OCT 28 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: OCT 28 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: OCT 28 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

Primary Reviewer: Eric B. Lewis, Oak Ridge National Laboratory
EPA Reviewer: Shannon Borges, Zigfridas Vaituzis, OPP/BPPD

STUDY TYPE:	Non-target Insect Testing (885.4340)
MRID NO:	47444913
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:	MSL0021036
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:	Evaluation of Potential Dietary Effects 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)
AUTHORS:	Paradise, M.A., C. Jiang, and D.B. Carson
STUDY COMPLETED:	January 24, 2008
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated compliance statement was provided. The study meets the requirements of 40 CFR Part 160 with the following exceptions: 1) the reference substance was not characterized by the sponsor under GLP standards, but a certificate of analysis was obtained, and 2) homogeneity and stability of the pollen of the control and reference substances in the diet treatments were not characterized in accordance with GLP standards.
STUDY SUMMARY:	A 16-day test was conducted to determine the dietary effects of pollen from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 corn on survival and development of the ladybird beetle (*Coleomegilla maculata*). Larvae were fed prepared diets containing either the test pollen, a control pollen from a conventional corn, or the control pollen plus a reference control of potassium arsenate. There were no statistically significant differences in survival, development, or growth of *C. maculata* larvae fed the test pollen or control pollen diets. The response of larvae fed the reference control diet was appropriate. Western blot analysis confirmed that the test pollen was present, homogeneous, and stable in the test diet.

45

CLASSIFICATION: Acceptable

Product Description

MON 89034 x TC1507 x MON 88017 x DAS-59122-7 is a combined trait corn that confers insect resistance and tolerance to glyphosate and glufosinate herbicides. MON 89034 produces Cry2Ab2 and Cry1A.105 to protect against feeding damage by lepidopteran pests. TC1507 produces Cry1F to control ECB larvae and other lepidopteran pests, as well as the phosphinothricin acetyl transferase (PAT) protein to confer tolerance to glufosinate-ammonium herbicides. MON 88017 produces Cry3Bb1 protein to protect against corn rootworm larvae, as well as the 5-enolpyruvylshikimate-3-phosphate synthase protein to confer tolerance to glyphosate herbicides. DAS-59122-7 produces Cry34Ab1 and Cry35Ab1 proteins to protect against coleopteran pests, as well as the PAT protein to confer tolerance to glufosinate-ammonium.

Test Material

MON 89034 x TC1507 x MON 88017 x DAS-59122-7 pollen, generated according to Monsanto production plan 06-01-52-06. When not in use, the pollen was frozen at approximately -80°C or on dry ice.

The control substance was pollen from conventional corn hybrid XE6001, which has a similar genetic background to the test substance. The control substance pollen was generated according to Monsanto production plan 06-01-52-06. When not in use, the pollen was frozen at approximately -80°C or on dry ice.

Prior to the test, the identity of the test and control pollens were confirmed using event-specific polymerase chain reaction, and the levels of the insect pest control proteins in the test substance pollen were determined using enzyme-linked immunosorbent assay.

The reference substance was potassium arsenate (Lot no. 056K1199, Sigma-Aldrich, St. Louis, MO).

Test Methods

The purpose of the 16-day test was to determine the dietary effects of pollen from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 on ladybird beetle (*Coleomegilla maculata*) larvae. Eggs were obtained from the USDA Agricultural Research Center in Beltsville, MD and hatched in Petri dishes. Newly-hatched first instars were cultured with corn earworm (*Helicoverpa zea*, CEW) eggs (Monsanto Co., Union City, TN) for approximately 24 hours.

Larval diets were prepared using pollen from the test substance, the control substance, or the control substance treated with potassium arsenate. All diets contained 50% w/w pollen and 50% w/w ground and lyophilized corn earworm eggs. To prepare the reference diet, pollen from the control substance was treated with potassium arsenate at 1000 mg/kg of pollen. Approximately 2 g of the treated pollen and 2 g of CEW eggs were mixed thoroughly to provide a final potassium arsenate concentration of 500 mg/kg of diet. Aliquots of each batch of diet were maintained in sealed containers placed inside ziplock bags and stored at approximately -20°C when not in use.

The test arenas consisted of an inverted 60 x 15 mm Petri dish with the lid as the base and the smaller dish as the top cover. Each test arena contained a 25-mm square cover glass (Corning, Acton, MA) sitting on a 55-mm diameter No. 1 filter paper (Whatman International, Ltd., Maidstone, England). The appropriate diet and an approximately 1-cm cotton roll saturated with purified water were placed on the cover glass. The contents of the test arenas were renewed at each feeding.

One first-instar larvae (24-48 hours old) was impartially placed in each test arena and allowed to feed *ad libitum*. Approximately 5 mg of the appropriate diet was provided every 48 hours, using a micro spoon. The test arenas were maintained at a temperature of 27°C, relative humidity of 70%, and a photoperiod of 14 hours light:10 hours darkness.

Each treatment was replicated three times with 25, 27, and 28 larvae, respectively, for a total of 80 larvae exposed to each diet. The larvae for each replicate originated from the same egg batch. The larvae were monitored every 48 hours for survival. Larvae that developed to the pupal stage were monitored every 24 hours for emergence as adults. Adults were weighed within 30 hours of eclosion using an analytical balance, and were sexed.

Samples (2 g) of freshly-prepared (Day 0) test substance and control substance diets were taken from the top, middle, and bottom of the mixing container and frozen at approximately -80°C. For storage stability analysis, an additional sample was collected from the test substance and control substance diets and frozen at approximately -20°C with the feeding aliquots of the diets until test end. After the 16-day test, the storage stability samples were removed from the -20°C storage and frozen at approximately -80°C until analysis.

The diet treatments were compared using statistical analysis (SAS version 9.1.3) with a significance level of 5%. Means were obtained for each replicate of each diet treatment, and the means and standard errors were calculated for each treatment across replicates. The SAS procedure PROC GLIMMIX was used to determine significant differences among the treatments for survival and the percent of larvae that developed to adults. Analysis of variance was used to determine significant differences in adult biomass of insects fed the test or control diets.

Results Summary

The levels of the insect control proteins in the pollen from the test substance determined prior to the test are given in Table 1.

TABLE 1. Cry protein levels in pollen of MON 89034 x TC1507 x MON 88017 x DAS-59122-7

Cry protein	Concentration (ng/mg fresh wt)[a]
Cry3Bb1	6.15 ± 0.498
Cry34Ab1	92.0 ± 2.51
Cry35Ab1	ND
Cry2Ab2	0.344 ± 0.007
Cry1A.105	9.05 ± 0.350
Cry1F	20.2 ± 1.65

[a]Mean of three replicates
ND = not detected at LOD of 0.03 ng/mg
Data from p. 23, MRID 47444909

47

Cry3Bb1 protein was used as an indicator of the amount of pollen in the test diets. Western blot analysis confirmed that Cry3Bb1 was present in the Day 0 test diet at a minimum of the 50% target level, was homogeneous in the test diet, and was stable during the freezer storage period. These analyses indicated that pollen was present at concentrations of 66% - 97% in the diet. Absence of Cry3Bb1 in the control substance diet was confirmed.

Results of the bioassay are summarized in Table 2. There was no significant difference in the survival of *C. maculata* larvae fed the test or control diet. Survival of larvae fed the reference substance was significantly lower than that of the other two groups. There was no significant difference in the development of larvae to adults between the test diet and control diet groups. No larvae fed the reference diet developed to adults. There was no significant difference in the adult biomass of the test diet and control diet groups.

TABLE 2. Survival and development of *C. maculata* after dietary exposure of larvae to test and control pollen diets

Treatment	No. of replicates	Total no. of insects	Mean survival (%)[a]	Mean % adults [a]	Mean adult biomass (mg)
MON 89034 x TC1507 x MON 88017 x DAS-59122-7	3	80	97.57 ± 1.21	96.34 ± 2.14	14.3 ± 0.18
Control	3	79	97.43 ± 1.29	97.43 ± 1.29	14.2 ± 0.03
Reference control	3	80	12.55 ± 3.30	0	--

[a]16 days after test start
Data from pp. 24-26, MRID 47444909

Study Authors' Conclusions

The study authors concluded that exposure to pollen from MON 89034 x TC1507 x MON 88017 x DAS-59122-7 at a concentration of 50% w/w in the diet had no adverse effect on the survival, development, or growth of *C. maculata.*

EPA Reviewer's Conclusion

Based on the data presented, exposure to MON 89034 x TC1507 x MON 88017 x DAS-59122-7 pollen at the 50% target concentration in the diet (concentrations actually ranged from 66 – 97%) did not result in adverse effects on *C. maculata.* These data confirm that the six Cry proteins stacked and produced in one plant do not pose any hazard to the ladybird beetle (*C. maculata*), a representative beneficial Coleopteran species.

48