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

OFFICE OF PREVENTION
PESTICIDES AND TOXIC
SUBSTANCES

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MEMORANDUM

SUBJECT Environmental Risk Assessment for modified Cry3A (mCry3A) *Bacillus thuringiensis* protein and the genetic material necessary for its production in Event MIR604 corn. Studies covered in this assessment have the following MRID Nos.: 462656-01 to 462656-15, 462656-17, 461556-01, 461556-03, and 461556-18.

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ENVIRONMENTAL ASSESSMENT

Background

Syngenta Ltd. has requested a registration for *Bacillus thuringiensis* mCry3A protein and the genetic material, which includes the PMI inert marker gene, necessary for its production in all corn lines and varieties. This protein is intended to control corn rootworm (CRW, *Diabrotica* spp.), a primary pest of corn in the United States. Corn rootworm larvae feed on corn roots, resulting in lodging and a reduction in a plant's ability to absorb water and nutrients from soil. In areas where the CRW is a pest (*e.g.* Corn Belt), significant financial losses are realized from decreased corn yields and increased expenditures on chemical pest control agents, including organophosphate, carbamate and pyrethroid insecticides.

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The EPA has conducted an environmental risk assessment of mCry3A when expressed in corn. General topics covered in this assessment include effects on wildlife, gene flow to related wild plants and its potential effects, and fate of mCry3A protein in the environment. This assessment is based on data submitted to EPA during the development of Event MIR604 corn lines, additional data submitted for registration, Federal Insecticide Fungicide and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) recommendations, consultations with scientific experts, and public comments on Plant-Incorporated Protectant (PIP) regulation.

A. Environmental Hazard Assessment

I. The Hazard Assessment Process

The EPA uses a tiered (Tiers I-IV) testing system to assess the toxicity of a Cry protein (*Bt* endotoxin) to representative non-target organisms that could be exposed to the toxin in the field environment. Tier I studies reflect a maximum hazard approach to testing, where single species are evaluated, generally in a laboratory setting, and mortality is the end point. Tiers II – IV generally encompass longer term greenhouse or field testing, and are implemented if adverse effects are seen at the Tier I level.

This tiered maximum hazard dose approach to testing was developed for the EPA by the American Institute of Biological Sciences and confirmed, in 1996, as an acceptable method of ecological hazard assessment by a FIFRA Scientific Advisory Panel on microbial pesticides and microbial toxins. The system was later accepted by the December 9, 1999 SAP for use with PIPs; however, this panel recommended that, for PIPs with insecticidal properties, additional testing of beneficial invertebrates closely related to target species and/or likely to be present in GM crop fields should be conducted. In October 2000, another SAP recommended that field testing should be used to evaluate population-level effects on non-target organisms. The August 2002 SAP, and some public comments, generally agreed with this approach, with the additional recommendation that indicator organisms should be selected on the basis of potential for field exposure to the subject protein. The December 1999 and August 2002 SAPs, and several public comments, also noted that the maximum hazard approach to non-target species testing was not statistically appropriate for determination of the No Observed Effect Level (NOEL). The Agency's OPPTS Testing Guidelines are in agreement with these comments.

Testing methods which utilize the tiered approach were last published as the Harmonized OPPTS Testing Guidelines, Series' 850 and 885 (EPA 712-C-96-280, February 1996). These guidelines, as defined in 40 CFR 152.20, apply to microbes and microbial toxins when used as pesticides, including those that are naturally occurring, and those that are strain-improved, either by natural selection or by deliberate genetic manipulation (mCry3A protein in corn, being a bacterial toxin, is covered by these testing guidelines).

The Guidelines call for testing of a single group or several groups of test animals at the maximum hazard dose level. When there is only one treatment group, at least 30 animals

must be tested at that treatment level. When there are multiple treatment groups, each shall contain at least 10 test animals. The guidelines further state that the duration of all Tier I tests should be approximately 30 days. Some test species, notably non-target insects, may be difficult to culture and the suggested test duration has been adjusted accordingly. Control and treated insects should be observed for at least 30 days after dosing, or in cases where an insect species cannot be cultured for 30 days, until negative control mortality rises above 20 percent.

The maximum hazard dose approach to environmental risk assessment is based on a safety factor times the maximum amount of active ingredient expected to be available to terrestrial and aquatic plants and animals in the environment (the expected environmental concentration, or EEC). Therefore, data that establishes an LC_{50}, ED_{50}, or LD_{50} that is greater than the maximum hazard dosage level (*e.g.* LD_{50} >10 X EEC) is sufficient to evaluate adverse effects, making lower dose testing unnecessary. If the LD_{50} is less than the maximum hazard test dose used in the Tier I evaluation, additional testing with sequentially lower doses is required to establish a definitive LD_{50}. The OPPTS Harmonized Guidelines call for testing at incrementally lower doses in order to quantify the hazard. The number of doses and test organisms evaluated must be sufficient to determine an LD_{50} value and, when necessary, the Lowest Observed Effect Concentration (LOEC), NOEL, or reproductive and behavioral effects such as feeding inhibition, weight loss, etc. In the final analysis, a risk assessment is made by comparing the LOEC to the EEC; when the EEC is lower than the LOEC, a no risk conclusion is made. Appropriate statistical methods, and appropriate statistical power, must be employed to evaluate the data.

The tiered approach to test guidelines ensures, to the greatest extent possible, that the Agency requires the minimum amount of data needed to make scientifically sound regulatory decisions. The EPA believes that maximum hazard dose Tier I testing presents a reasonable approach for evaluating hazards related to the use of biological pesticides and for identifying negative results with a high degree of confidence. The Agency expects that Tier 1 testing for short-term hazard assessment will be sufficient for most studies submitted in support of plant-incorporated *Bt* Cry protein registrations. However, if long range adverse effects must be ascertained, then higher-tier longer-term field testing will be required. As noted above, the October 2000 SAP and the National Academy of Sciences (NAS 2000) recommended testing non-target organisms directly in the field. This approach, with an emphasis on testing invertebrates found in corn fields, was also recommended by the August 2002 SAP and was supported by several public comments.

Bacillus thuringiensis Cry endotoxins are proteins and proteins do not bioaccumulate. The biological nature of protein makes *Bt* Cry toxin readily susceptible to metabolic, microbial, and abiotic degradation once they are ingested or excreted into the environment. Although there are reports that Cry protein binds to soil particles, it has been shown that these proteins are degraded rapidly by microbes upon elution from soil. The same sources also report that *Bt* proteins present in soil collected from *Bt* corn fields have no detectable adverse effect on soil invertebrates or culturable microbial flora.

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Since delayed adverse effects and/or accumulation of toxins through the food chain are not expected to result from exposure to *Bt* Cry proteins, these protein toxins are not routinely tested for chronic effects on non-target organisms.

II. Non-Target Wildlife Hazard Assessment

Two separate SAP reports (October 2000 and August 2002) recommended that non-target testing of *Bt* Cry proteins should focus on invertebrate species exposed to the crop being registered. Following SAP recommendations, the EPA determined that non-target organisms with the greatest exposure potential to Cry protein in transgenic corn fields are beneficial insects, which feed on corn pollen and nectar, and soil invertebrates, particularly Coleoptera species. Therefore, maximum hazard dose toxicity testing on representative beneficial organisms from several taxa was performed in support of this Section 3 FIFRA registration. The toxicity of the mCry3A protein has been evaluated on several species of invertebrates including the lady beetle, Carabid beetle, rove beetle, flower bug, honey bee, and earthworm. Reproductive and developmental observations were also made in the lady beetle, rove beetle and honeybee studies.

Although the mCry3A protein is known to be very host specific, conferring toxic effects on corn rootworm, Colorado potato beetle, and closely related species, and despite the October 2000 and August 2002 SAP's recommendations against testing of non-target species not related to susceptible target pests, EPA has done a risk assessment on a range of non-target wildlife to comply with the Agency's published non-target data requirements (in the absence of PIP-specific risk assessment guidance, EPA requires applicants for PIP registrations to meet the 40 CFR Part 158 data requirements for microbial toxins). These requirements include birds, mammals, plants and aquatic species. In addition, an earthworm study was voluntarily submitted to the Agency to ascertain the potential effects of mCry3A on beneficial decomposer species.

Test substances (*i.e.* source of mCry3A protein) used for studies submitted in support of the mCry3A registration included bacterially-produced purified mCry3A protein (referred to as mCry3A-0102) and corn grain. The October 2000 SAP recommended that while actual plant material is the preferred test material, bacterially-derived protein is also a valid test substance, particularly in scenarios where test animals do not normally consume corn plant tissue and where large amounts of Cry protein (Cry protein concentrations that exceed levels present in plant tissue) are needed for maximum hazard dose testing. An insect feeding study, which compared the relative potency of plant produced mCry3A protein to microbe produced mCry3A-0102, indicated that plant produced protein was twice as toxic as microbe produced protein (see Section A.II.1.d.vii). However, since exposure to mCry3A-0102 was at least several times the EEC for all submitted non-target studies, EPA determined that exposure to mCry3A protein was adequate despite the lower potency of microbe produced mCry3A-0102. In accordance with OPPTS Harmonized Testing Guidelines, adult insect studies were generally conducted for 30 days or until mortality in the negative control reached, or exceeded, 20% and larval studies were carried out through pupation and adult emergence.

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The results of ecological effects studies submitted in support of the MIR604 Section 3 FIFRA registration are summarized in Table 1 and presented in a more descriptive format in subsequent sections of this risk assessment document. Full reviews of each study can be found in the individual Data Evaluation Reports (DERs) and accompanying memos.

Table 1. Summary of environmental effects studies and waiver justifications submitted to EUP to comply with data requirements published in 40 CFR § 158.740.

Guideline	Study	Results	MRID
885.4150	Wild Mammal Testing, Tier I	Mammalian wildlife exposure to mCry3A protein is considered likely; however, mCry3A-0102 toxicity data indicate that, when tested at the maximum hazard dose level, there was no significant toxicity to rodents. Therefore no hazard to mammalian wildlife is anticipated and data on wild mammal testing is not required.	N/A
850.2100	Avian Acute Oral Toxicity Test, Tier 1 (Northern Bobwhite quail, *Colinus virginianus*)	Young adult northern bobwhite quail were administered a single nominal oral dose of 722 mg mCry3A-0102/kg body wt and observed for 14 days. There were no treatment-related adverse clinical signs or mortality. Body weight and feed consumption of the test birds were comparable to those of the controls. Classification: Supplemental	461556-16
885.4050	Avian Oral, Tier 1 (Broiler, *Gallus domesticus*)	In a 49-day feeding study, commercial broiler chickens were fed formulated diets containing one of the following ingredients: MIR604 corn (contained mCry3A protein); an MIR604 isoline (no mCry3A protein); or a non-transgenic commercial corn hybrid (no mCry3A protein). No adverse clinical signs were noted, and carcass yield and mortality were not significantly different among treatment groups. Classification: Acceptable	462656-15
885.4200	Freshwater Fish Testing, Tier 1 (Rainbow trout, *Onchorhynchus mykiss*)	In a 28-day toxicity study, granular fish feed containing 50% by weight Event MIR604 corn grain did not produce statistically significant mortality or sublethal effects when fed twice daily to juvenile rainbow trout. Classification: Acceptable	461556-17 462656-02
885.4280	Estuarine and Marine Animal Testing, Tier I	Estuarine and marine animal studies are not required for this product, because mCry3A is not intended for direct application to estuarine or marine environments and there is very low potential that these ecosystems will be exposed to mCry3A protein in field corn.	N/A
885.4300	Nontarget Plant Studies, Tier I	The active ingredient is an insect toxin (*Bt* endotoxin) that is non-toxic to aquatic and terrestrial plants. Consequently, non-target plant studies have been waived for this product.	N/A
885.4340	Nontarget Insect Testing, Tier 1 (Lady beetle, *Coccinella septempunctata*)	Lady beetle larvae were fed live pea aphids that were dipped in a solution containing: 50µg mCry3A-0102/mL Agral 90 solution (a non-ionic surfactant); Agral 90 solution only (negative control); or 0.5 mL of Nemolt (teflubenzuron)/L Agral 90 solution (positive control). The rate of pupal development was not significantly different between the negative control and mCry3A-0102 treatments. However, the number of days to adult emergence was significantly lower in the mCry3A-0102 treatment. Classification: Acceptable	462656-03 462656-04

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Guideline	Study	Results	MRID
885.4340	Nontarget Insect Testing, Tier 1 (Carabid beetle, *Poecilus cupreus*)	Twenty-four to 48 hour-old Carabid beetle larvae were fed daily until pupation with blowfly pupae that had been injected with one of three treatments. Results showed no significant difference, in the percent of pre-imaginal mortality or mean weight of emerged adults, between the mCry3A treatment and the negative control group. Classification: Acceptable	462656-05 462656-06
885.4340	Nontarget Insect Testing, Tier 1 (Rove beetle, *Aleochara bilineata*)	Rove beetles were provided approximately 0.2 g of minced beef treated with: 50 µg mCry3A protein/g meat; 10 mL deionized water/90 g meat (negative control); teflubenzuron at a rate of 0.01 mg a.i./g meat (positive control). Beetle mortality and reproductive capacity were not adversely affected by feeding on a test diet composed of 45.85 µg mCry3A-0102/g diet for 35 days. Classification: Acceptable	462656-07 462656-08
885.4340	Nontarget Insect Testing, Tier 1 (Insidious flower bug, O*rius. insidiosis*)	Nymphal flower bugs were fed, on a daily basis, diet with one of three treatments: 50 µg mCry3A-0102 /g of diet; 20 mL deionized water/per 180 g diet (negative control); or teflubenzuron at a rate of 0.01 mg a.i./g diet. Mortality in the mCry3A-0102, negative control, and positive control treatments were 18, 23, and 98%, respectively. Average development time for all treatments was not significantly different. Classification: Acceptable	462656-09 462656-10
885.4380	Honey Bee Testing, Tier 1 (*Apis mellifera)*	Honeybees were exposed to sucrose solution containing 50 µg of the test material/g sucrose solution, or a positive or negative control. Results suggest that incidental ingestion of mCry3A proteins would not adversely affect the hive condition, survival of larvae in brood cells, or exposed adult worker bees. Classification: Acceptable	461556-18
885.4240	Aquatic Invertebrate Acute Toxicity Test, Tier 1	The only plausible potential route of exposure of freshwater invertebrates to insecticidal proteins produced by transgenic corn plants is corn pollen drift into aquatic habitats. However, since the pollen of Event MIR604 corn plants has no detectable mCry3A protein, exposure of freshwater aquatic invertebrates to mCry3A protein will be negligible. Classification: Acceptable	Waiver justification
850.620	Earthworm Subchronic Toxicity Study (*Eisenia fetida)*	Earthworms were exposed to soil containing mCry3A-0102 at a nominal concentration of 370 µg/g dry soil for 14 days, or one of two control treatments. At test end, a mortality rate of 5% and a mean weight loss of 5.8% were recorded for mCry3A-0102 treated worms. For the negative control, mortality was 0%, and mean weight loss was 11.4%. Mortality was 100% for the positive control. Classification: Supplemental	462656-11 462656-12
N/A	Insecticidal Activity Spectrum Study	The mCry3A protein has a similar spectrum of activity to native Cry3A, but with enhanced toxicity to NCRW and WCRW. Modified Cry3A produced in *E. coli* and maize were found to be active against WCRM with 144 hour LC_{50} values of 0.43 µg mCry3A-0102/mL diet and 0.20 µg mCry3A/mL diet surface, respectively. The insecticidal toxin potencies of the two proteins differ by a factor of two. Classification: Acceptable	461556-01 461556-03
885.5200	Expression in a Terrestrial Environment (Soil Fate)	A simple first-order kinetic model, based on CPB larvae feeding data, determined that the DT_{50} for mCry3A-0102 in this silty clay loam soil was 7.6 days. This finding suggests that soil incorporated binary insecticidal protein degrades over time. Classification: Supplemental	462656-14

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Guideline	Study	Results	MRID
NA	Environmental Fate Assessment	MIR604 corn plants have been shown to express mCry3A protein in leaves, kernels, roots, and silks, but the protein was not detected in corn pollen. Due to corn's lack of invasive characteristics and the low probability that the *mCry3A* gene from Event MIR604 would transfer to a wild relative of corn, it is unlikely that mCry3A will spread beyond cultivated sites and persist in weedy populations. It is also unlikely that genes present in MIR604 corn would be subject to horizontal gene transfer at a frequency that exceeds the rate of transfer in other plants. Classification: Supplemental	462656-13

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1. Non-target Wildlife Testing and Hazard Assessment

a. Mammalian Wildlife

Mammalian wildlife exposure to mCry3A protein is considered likely; however, mammalian toxicology information gathered to date on *Bt* Cry proteins does not show a hazard to wild mammals. And an acute oral toxicity test, submitted to EPA in support of the MIR604 registration (see Human Health Risk Assessment), indicated that no significant toxicity was seen when rodents were exposed to mCry3A at the maximum hazard dose level. Therefore, no hazard to mammalian wildlife is anticipated and data on wild mammal testing is not required for this registration.

b. Avian hazard assessment

Published data and studies on file at EPA show that consumption of *Bt* corn has no measurable deleterious effects on avian species. However, to comply with published data requirements, the following studies were submitted to EPA in support of the MIR604 product registration. These studies were GLP compliant and, when considered together, meet EPA data requirements for avian species.

i. Northern Bobwhite Quail (MRID 461556-16)

This study meets current EPA Guideline requirements for acute toxicity testing of incidental exposures of plant incorporated Cry proteins to non-target birds in the wild.

Young adult (25 week old) northern bobwhite quail (*Colinus virginianus*) were administered a single nominal oral dose of 722 mg mCry3A-0102/kg body wt and observed for 14 days. There were no adverse treatment-related clinical signs or mortality. Body weight and feed consumption of the test birds were comparable to those of the negative control. The acute oral LD_{50} of mCry3A-0102 was shown to be greater than a nominal concentration of 722 mg mCry3A-0102/kg body wt (approximately 652 mg mCry3A protein/kg body wt). These data show that there will be no adverse effects on avian wildlife from incidental field exposure to mCry3A corn.

ii. Broiler study (MRID 462656-15)

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The submitted study was not EPA GLP compliant, but was conducted according to accepted scientific methods.

In a 49-day avian feeding study, one day-old commercial broiler chickens (*Gallus domesticus*, Ross 344 males and feather-sexable Ross 308 females) were fed formulated diets containing one of the following ingredients: MIR604 corn (contained mCry3A protein); an MIR604 isoline (no mCry3A protein), or a non-transgenic commercial corn hybrid (no mCry3A protein). Starter, grower (days 16-31), and finisher diets (days 31-49) contained 57.5, 63.0 and 67.5% corn, respectively. The concentration of plant produced mCry3A in the transgenic diets was reported to be 0.04, 0.06, and 0.08 µg/g dry weight of the starter, grower, and finisher diets, respectively. Chicks were separated by sex and placed into single sex pens containing 25 birds each. Each treatment group contained 6 cages each of males and females (12 cages x 25 birds/cage = 300 birds). Pen weights (25 birds/pen) were recorded at days 1 (hatch), 16, 31, and 49. On the later three dates, feed conversion ratios were determined. Feeding was terminated approximately16 hours before slaughter on day 51. Body weight, feed conversion, and survival data were recorded. Results indicate that sex had a significant effect on body weight and survival, with males weighing more and having higher mortality. No adverse clinical signs were noted, and carcass yield and mortality were not significantly different among treatment groups.

Table 2. Mean body weight of broiler chickens fed mCry3A positive or mCry3A negative corn grain.

Treatment	Body Weight (g)			
	Day 1 (hatch)	Day 16	Day 31	Day 49
MIR604 (mCry3A positive)	44.60 ± 0.19 a*	547.8 ± 9.6 a	1684.1 ± 50.6 a	3468.4 ± 129.5 a
MIR604 (mCry3A negative)	44.66 ± 0.18 a	563.0 ± 5.4 a	1690.5 ± 40.2 a	3469.4 ±113.1 a
Commercial hybrid (mCry3A negative)	44.70 ± 0.19 a	551.1 ± 9.6 a	1634.0 ± 44.3 b	3365.2 ± 117.6 b

* Within sampling days, means followed by different letters differ significantly ($p \leq 0.05$).

Table 3. Carcass and parts yield at day 51 for broiler chickens fed mCry3A positive or mCry3A negative corn grain.

Males				
Treatment	Dressed carcass*	Thighs	Pectoralis major	Pectoralis minor
	---------- g ----------			
MIR604 (mCry3A positive)	2947.2 ± 57.44 a**	526.3 ± 14.05 a	638.8 ± 19.18 a	150.8 ± 4.63 a
MIR604 (mCry3A negative)	2936.7 ± 56.88 a	484.3 ± 15.10 ab	609.6 ± 16.73 a	145.7 ± 4.57 a
Commercial hybrid (mCry3A negative)	2812.9 ± 61.49 a	454.6 ± 20.30 b	605.1 ± 23.09 a	151.3 ± 4.97 a
Females				
MIR604 (mCry3A positive)	2248.3 ± 45.53 a	380.8 ± 7.6 a	515.4 ± 18.9 a	126.2 ± 3.3 a
MIR604 (mCry3A negative)	2291.0 ± 41.4 a	361.9 ± 9.2 a	511.8 ± 13.8 a	128.0 ± 3.5 a

Commercial hybrid (mCry3A negative)	2204.2 ± 54.8 a	366.2 ± 11.3 a	487.1 ± 22.0 a	125.9 ± 3.4 a

* Fresh carcass without head, neck, feet, feathers, viscera, and blood
** Within carcass parts categories, means followed by different letters differ significantly ($p \leq 0.05$)

c. Aquatic species testing

There is no evidence for sensitivity of aquatic (including endangered) species to anti-coleopteran *Bt* Cry proteins. Furthermore, aquatic exposure to mCry3A is extremely small or non-existent since mCry3A is not expressed in MIR604 pollen.

i. Freshwater Fish (MRIDs 461556-17 and 462656-02)

The Harmonized Testing Guidelines requirement for a static renewal freshwater fish toxicity study is usually waived for *Bt* corn PIPs due to the low potential for exposure to Cry protein produced in this crop. Nonetheless, a 28 day flow-through study was performed and submitted for review. This study is scientifically sound.

In this 28-day toxicity study, juvenile rainbow trout (*Onchorhynchus mykiss*) were fed fish feed containing 50% w/w Event MIR604 (0.09 µg mCry3A/g test diet) or non-transgenic (negative control) corn grain. Prior to test initiation, 40 fish were placed in each of two test vessels, the exposure tank and the control tank. Mortality and symptoms of toxicity were assessed on a daily basis and detailed observations of symptoms and feeding responses were made on days 4, 7, 10, 15, and 22. No significant differences were detected in the weight of the control or test fish at 0, 14, or 28 days. No significant difference in length was seen at 14 or 28 days. In the MIR604 test group, transient discoloration, sounding, and surfacing were seen in one to three fish after day 15, and one fish (2.5% of test group) was found dead on day 21. No mortality was seen in the control group. Due to the lack of demonstrated toxicity of the mCry3A protein to juvenile rainbow trout and the low probability that aquatic systems will be exposed to the protein, no fresh water fish hazard is expected from commercial cultivation of Event MIR604 corn.

ii. Aquatic invertebrates

The only plausible potential route of exposure of freshwater invertebrates to insecticidal proteins produced by transgenic corn plants is corn pollen drift into aquatic habitats. However, since the pollen of Event MIR604 corn plants has no detectable mCry3A protein, exposure of freshwater aquatic invertebrates to mCry3A protein will be negligible.

iii. Estuarine and Marine Animals

Estuarine and marine animal studies were not required for this product, because of the low probability that aquatic systems will be exposed to the mCry3A protein produced in MIR604 corn plant tissues.

iv. Terrestrial and Aquatic Plants

Plant toxicity studies were not required for this product because the active ingredient is an insect toxin (*Bt* endotoxin) that has never shown any toxicity to plants.

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d. Non-Target Insect Testing

The mCry3A protein specifically targets corn rootworm species, which are within the order Coleoptera (beetles). Since *Bt* toxins are known to have a limited host range, EPA requires that test species used for non-target insect evaluations should include several species that are related to the target pests (coleopteran species), since it is expected that these species will be most susceptible to the *Bt* toxin.

i. Ladybird Beetle (MRIDs 462656-03 and 462656-04)

This study complies with the testing requirements outlined in OPPTS Series 885.4340 (Nontarget Insect Testing, Tier 1), the Organization for Economic Development (OECD) Principles of Good Laboratory Practice, and the UK Good Laboratory Practice Regulations.

Four–day old lady beetle larvae (*Coccinella septempunctata*) were fed live pea aphids that were dipped in one of three solutions for a period of 14 days. For each treatment, aphids were immersed for 30 seconds in a solution containing: mCry3A dissolved in a solution of Agral 90 (a non-ionic surfactant) at a concentration of 50 µg mCry3A-0102 protein/mL solution; Agral 90 solution only (negative control); or 0.5 mL of Nemolt (teflubenzuron)/L Agral 90 solution (positive control). Freshly-treated live aphids were provided to lady beetle larvae daily until pupation, and the number of pea aphids provided at each feeding time increased with larval age. Beetle larvae were assessed daily for developmental stage and mortality. Following adult emergence from pupae, beetles were fed 50 treated aphids 3 times a week for 14 days. Adults were assessed for mortality at each feeding

There was no significant difference in the rate of pupal development between the negative control and mCry3A-0102 treatment. However, the mean number of days to adult emergence was significantly lower in the mCry3A-0102 treatment (Table 4). There was no significant difference in pre-imaginal or adult survival among the negative control and mCry3A-0102 treatments. All larvae in the positive control died in the pre-imaginal stage (Table 5).

Table 4. Mean developmental time for lady beetles exposed to mCry3A-0102 or a control treatment.

Treatment	Concentration	Larvae to Pupae		Larvae to Adult	
		Mean	Standard Deviation	Mean	Standard Deviation
Negative control	Agral 90	5.48	0.50	9.8	0.68
mCry3A-0102	50 µg mCry3A/mL Agral solution	5.33	0.47	9.48*	0.59

*Significantly different from control p>0.05

Table 5. Mortality assessment for lady beetles exposed to mCry3A-0102 or a control treatment.

Treatment	Concentration	Pre-Imaginal Mortality	Adult Mortality

		Mortality	Corrected Mortality	Mortality	Corrected Mortality
		---------- % ----------			
Negative control	Agral 90	0	-	7.5	-
mCry3A-0102	50 µg mCry3A/mL Agral solution	0	0	15.0	8.1
Nemolt	0.5 mL/L Agral solution	100*	100	-	-

* Treatment differed significantly from the negative control ($P < 0.001$)

It is noted that the actual amount of mCry3A-0102 protein consumed by larvae and adult ladybird beetles was 9 µg mCry3A-0102/g aphid, which is below the targeted concentration of 50µg/g aphid (10 x mCry3A concentration in corn leaves). However, since lady beetles are known to feed on corn pollen or insect prey, rather than corn leaves, and since mCry3A concentration in pollen (undetected) and prey is much lower than in corn leaves, it is not expected that lady beetles will be adversely affected by MIR604 corn in a field environment.

ii. Carabid Beetle (MRIDs 462656-05 and 462656-06)

This study complies with the testing requirements outlined in OPPTS Series 885.4340 (Nontarget Insect Testing, Tier 1), the Organization for Economic Development (OECD) Principles of Good Laboratory Practice, and the UK Good Laboratory Practice Regulations.

Twenty-four to 48 hour-old Carabid beetle larvae (*Poecilus cupreus*) were fed daily with blowfly pupae until pupation. For each treatment, blow fly pupae were injected with: mCry3A-0102 at a rate of 50µg mCry3A/g pupa; deionized water only (negative control); or teflubenzuron at a rate of 0.664 ng a.i./g fly pupae (positive control). Fly pupae were replaced daily with freshly defrosted pupae until beetle larvae entered pupation. Beetle larvae were assessed three times per week for the first two weeks, and two times per week thereafter until day 32 of the study, at which time test containers were checked daily for emerging adult beetles. Adults were sexed, and overall weight and mortality were statistically analyzed. Results showed no significant difference, in the percent of pre-imaginal mortality or mean weight of emerged adults, between the mCry3A treatment and the negative control group. The positive treatment resulted in 100% pre-imaginal mortality (Table 6).

Table 6. Larval mortality and adult weight of Carabid beetles fed blowfly pupae treated with or without mCry3A-0102 protein.

Treatment	Percent Larval Mortality	Percent Corrected Larval Mortality	Mean Adult Weight
mCry3A-0102	10	0	82.9
Negative control	20	-	81.5
Positive control	100	100	-

Analysis of the test diet showed that the actual concentration of mCry3A in blowfly pupae was 12 µg/g pupae. This amount is less than 10X the expressed concentration in maize leaves (50 µg). However, the most likely route of Carabid exposure to mCry3A

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protein is through consumption of prey that has eaten MIR604 plant tissue and studies indicate that the concentration of mCry3A in prey is at least 1.4X lower than the protein concentration in plant tissue.

iii. Rove Beetle (MRID 462656-07 and 462656-08)

This study complies with the testing requirements outlined in OPPTS Series 885.4340 (Nontarget Insect Testing, Tier 1), the Organization for Economic Development (OECD) Principles of Good Laboratory Practice, and the UK Good Laboratory Practice Regulations.

Rove beetles (*Aleochara bilineata*) were obtained from parasitized onion fly (*Delia antique*) pupae and adults were four days old (physiologically) at study initiation. During this 35 day feeding trial, beetles were provided one of three treatments of cooked minced beef. For each treatment, beetles were given approximately 0.2 g of minced beef treated with: 50 µg mCry3A protein/g meat; 10 mL deionized water/90 g meat (negative control); or teflubenzuron at a rate of 0.01 mg a.i./g meat (positive control). For the first 7 days, beetles (10 female and 10 male) were kept in round plastic pots. From days 7 to 35, test arenas were comprised of polystyrene boxes filled with at least 4 cm of quartz sand. On days 1, 7 and 35 living, moribund, dead, and missing beetles were noted. To assess beetle fecundity, approximately 500 onion fly pupae were incorporated beneath the sand surface in each test box on days 14, 21, and 28. After approximately seven days, fly pupae were removed from the sand and placed in plastic pots. F_1 beetles emerging from onion fly pupae were recorded every 1 to 4 days through day 76. The study was concluded when the mean number of beetles emerging per replicate declined to less than two per day in the control treatment. The mean number of F_1 progeny was 647 for the negative control and 663 beetles for the mCry3A-0102 treatment and these results were not significantly different. At day 35, mortality in the mCry3A, negative treatment, and positive treatments were 31, 34, and 35%, respectively and did not differ significantly (Table 7). Results also indicate that the reproductive capacity of beetles feeding on the mCry3A-0102 test diet was not adversely affected.

Table 7. Mortality and number of progeny of rove beetles supplied mCry3A-0102 or a control treatment for 35 days.

Treatment	% Mortality at 35 days*	% Corrected Mortality	Mean no. of F_1 Progeny	% Effect on Reproduction
mCry3A-0102 (50 µg mCry3A/g diet)	31	0	663 ± 219	-2.5
Negative control	34	-	647 ± 169	-
Teflubenzuron (10 µg/g diet)	35	2	3 ± 5**	99.5

* Treatments did not differ significantly from the negative control ($p>0.05$)

** Treatment differed significantly from the negative control ($p<0.001$)

iv. Flower Bug (MRID 462656-09 and 462656-10)

This study complies with the testing requirements outlined in OPPTS Series 885.4340 (Nontarget Insect Testing, Tier 1), the Organization for Economic Development (OECD)

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Principles of Good Laboratory Practice and the UK Good Laboratory Practice Regulations.

Nymphal *Orius insidiosis* were fed, on a daily basis, diet consisting of cooked beef, liver, yeast, honey, egg, sugar, water, and Nipagin (chemical preservative) for 21 days. The diet was treated with one of three treatments: 50 µg mCry3A-0102 /g diet; deionized water (negative control); or teflubenzuron at a rate of 0.01 mg a.i./g diet. For each treatment, approximately 0.2 g of diet was placed into a small plastic cup covered with parafilm; insects pierced the parafilm to reach the diet. Nymphs were assessed for mortality and vitality daily until adulthood, or until 21 days after test initiation. Bugs that were missing, squashed, or injured during the study were excluded from data analysis. Mortality in the mCry3A-0102, negative control, and positive control treatments were 18, 23, 98%, respectively (Table 8). Average development time for all treatments was not significantly different ($p>0.5$). Analysis of the test diet showed that protein expression was 47.8 µg mCry3A/g diet, or 95.6% of the nominal concentration (50 µg/g diet). In addition, a bioassay in which Colorado potato beetle, CPB (*Leptinotarsa decemlineata*) larvae were fed a diet containing 10 or 20% mCry3A-0102-treated diet resulted in mortality of 83 and 90%, respectively. Although control mortality was 23% (test guidelines state that negative control mortality should not exceed 20%), it is unlikely that study conclusions were affected by this high rate of mortality.

Table 8. Mortality of flower bugs supplied with diet containing mCry3A-0102 or a control diet.

Treatment	% Pre-imaginal Mortality	% Corrected Mortality
mCry3A-0102	18	0
Negative control	23	-
Teflubenzuron (10 µg/g diet)	98*	97

* Significantly different from negative control ($p<0.001$)

v. Honey Bee Larvae (MRID 461556-18)

An acceptable study was conducted based on OPPTS Series 885.4380 (Honey Bee Testing, Tier I), in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 160.

Honeybees (*Apis mellifera*) were exposed via in-hive feeders to sucrose solution containing 50 µg test material/g sucrose solution, a negative control of 50% w/v sucrose solution, or a positive control of 480 g/L diflubenzuron insect growth regulator (Dimilin Flo ™) in sucrose solution. Fresh treatment solutions were provided to each hive daily for five days. Egg cell mortality in the negative control, test, and positive control groups was 28.5, 27.3, and 100%, respectively. Larval cell mortality was 6.0, 6.8, and 100%, respectively. There was no significant difference in mortality between the test and negative control groups for cells with eggs or larvae. There was also no significant difference in pre- and post-test hive condition between the test and negative control

treatments. Results for the positive control treatment were significantly different from the other treatments for both mortality and hive condition (Table 9). Adult bees were not affected by any of the treatments. These results suggest that incidental ingestion of mCry3A proteins would not adversely affect the hive condition, survival of larvae in brood cells, or exposed adult worker bees.

Table 9. Honey bee egg and larval cell development and brood count per frame when exposed to mCry3A-0102 or a control treatment.

Treatment	Brood Development Assessments				Mean Brood per Frame	
	Egg Cells		Larvae Cells		Before Treatment	After Treatment
	Mortality	Corrected Mortality	Mortality	Corrected Mortality		
	----------%----------					
Negative control	28.5	-	6.0	-	36.4	49.6
mCry3A-0102	27.3	0	6.8	0.9	34.2	40.7
Dimilin Flo	100.0*	100.0	100.0	100.0*	34.5	27.7*

* Significantly different from negative control ($p < 0.01$)

vi. Freshwater Aquatic Invertebrate (waiver justification)

The only plausible potential route of exposure of freshwater invertebrates to insecticidal proteins produced by transgenic corn plants is corn pollen drift into aquatic habitats. However, since the pollen of Event MIR604 corn plants has no detectable mCry3A protein, exposure of freshwater aquatic invertebrates to mCry3A protein will be negligible.

vii. Earthworm Toxicity Testing (MRIDs 462656-11 and 462656-12)

This study complies with the testing requirements outlined in OPPTS Series 850.6200 (Earthworm Subchronic Toxicity Study), Good Laboratory Practice Standards as published by the EPA in 40 CFR Parts 160 and 792, and the Organization for Economic Development (OECD) Principles of Good Laboratory Practice.

In a laboratory test, earthworms (*Eisenia fetida*) were exposed to mCry3A-0102, incorporated into artificial soil, at a nominal concentration of 370 µg/g dry soil (334 µg mCry3A protein/g dry soil) for 14 days. A negative control of deionized water and artificial soil and a positive control of 10, 20, 30 40, or 50 mg 2-chloroacetamide/kg dry soil were also used in the test. At test end, a mortality rate of 5% and a mean weight loss of 5.8% were recorded for mCry3A-0102 treated worms (Table 10). Worms included in the negative control had a mortality rate of 0% and a mean weight loss of 11.4%. Positive control worms exposed to ≥30 mg 2-chloroacetamide/kg of dry soil had a mortality rate of 100% and the LC_{50} value for earthworms exposed to 2-chloroacetamide was approximately 18 mg active ingredient/kg dry soil. The 14-day LC_{50} for earthworms exposed to mCry3A-0102 in an artificial soil substrate was determined to be greater than 370 µg active ingredient/g dry soil (the highest concentration tested), or 67 times greater than the expected field concentration of 5.5 µg/g soil (based on mCry3A concentration in senescent MIR604 plant roots). These findings, which are consistent with historical *Bt* cry protein feeding results, indicate that earthworms should not be adversely affected by MIR604 corn plants.

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Although some public comments have questioned whether earthworm test organisms actually ingested the soil incorporated *Bt* Cry proteins, recently published data show that earthworms do ingest and excrete soil incorporated *Bt* Cry proteins.

Table 10. Mortality and weight loss of earthworms exposed to mCry3A-0102 or a control treatment.

Treatment	Cumulative mortality (%)		14-Day Weight loss (%)
	Day 7	Day 14	
mCry3A-0102	2.5	5.0	5.8
Negative control	0	0	11.4

viii. Insecticidal Activity Spectrum Study (MRIDs 461556-01 and 461556-03)

Insect susceptibility studies showed that native Cry3A is primarily active against Colorado potato beetle and has minimal activity against northern corn rootworm (NCRW); both species are members of the Chrysomelidae family of beetles. The mCry3A protein has a similar spectrum of activity to native Cry3A, but with enhanced toxicity to NCRW and western corn rootworm (WCRW).

The bioactivity of the recombinant *E. coli* and maize event MIR604 mCry3A proteins were compared using a diet incorporation bioassay with first instar WCRW. Modified Cry3A from both *E. coli* and maize proteins were found to be active against WCRW with 144 hour LC_{50} values of 0.43 µg mCry3A/mL diet and 0.20 µg mCry3A/mL diet surface, respectively (Table 11). The insecticidal toxin potencies of the two proteins differ by a factor of two.

Table 11. **Toxicity of mCry3A protein derived from recombinant *E. coli* (mCry3A-0102) or from corn event MIR604 on WCRW larvae.**

Sample	LC50	
	µg mCry3A protein/mL diet	95% Confidence Interval
LPMIR604-0103 (corn-derived protein)	0.20	(0.09 - 0.41)
mCry3A-0102 (bacterial-derived protein)	0.43	(0.14 - 0.94)

2. Soil Fate (MRID 462656-14)

Soil organisms may be exposed to mCry3A protein through contact with corn plant roots (by direct feeding), corn plant root exudates, incorporation of above-ground plant tissues into soil following harvest, or by soil-deposited pollen. Some evidence suggests that acidic soils (pH 5.6), and those which are high in clays and humic acids, are more likely to bind Cry protein, and thus decrease the rate of protein degradation by soil microorganisms. It is noted, however, that the pH factor should not contribute to protein binding in corn fields, since maize is generally grown on neutral soils (above pH 5.6). And despite evidence that soils high in clay and humic acids may bind cry proteins, and thus interfere with the microbial degradation processes, the weight of evidence suggests

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that Cry proteins do not accumulate in soil to arthropod-toxic levels. Nonetheless, the Agency requires soil fate evaluations for each new insect protected crop.

This study was conducted in accordance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160.

The test soil was a silty clay loam collected from a corn-growing region of Iowa. Treatments were the following: mCry3A test mixture (*Bt* cry protein and water) mixed with soil at a nominal dose of 230 µg mCry3A/g d/w soil, and sampled after 0, 1, 3, 7, 12, and 30 days of incubation; a negative control consisting of soil dosed with deionized water; and a positive control consisting of mCry3A-0102 mixed with test diet (no soil), at a concentration of 230 µg mCry3A/g diet (equivalent test dose level at Day 0).

For the CPB bioassay, mCry3A-treated soil collected at each incubation time point was incorporated into a stock diet at a concentration of 10% w/w, and the resulting suspension was poured into Petri dishes. Negative and positive controls were prepared in the same manner. Ten freshly-hatched CPB larvae were placed in each Petri dish, which were then covered and maintained under ambient laboratory conditions. Test material and negative control treatments were replicated 12 times (120 larvae/treatment) and the positive control was replicated four times (40 larvae). Larval mortality was assessed at 72 hours.

Mean CPB larval mortality in mCry3A-0102- treated soil ranged from 48-54% during the first week, then declined rapidly to 9% on Day 30 (Table 12). The DT_{50} value (time for 50% of initial bioactivity to dissipate) for degradation of mCry3A in this soil was estimated to be 7.6 days. Biomass determinations of soil at study start and end showed that microbial activity was maintained during the study.

Table 12. mCry3A protein bioactivity in treated soil as measured by CPB larvae mortality.

Treatment	% CPB Mortality
Negative control	18
Positive control	53
Test Treatment	
Day 0	53
Day 1	54
Day 3	48
Day 7	51
Day 12	11
Day 30	9

Based on these results, it may be concluded that purified mCry3A-0102 insecticidal proteins degrade rapidly in silty clay loam soil. However, silty clay loam soil is just one of many soil classes used for corn production in the United States. A more useful study would evaluate protein degradation, accumulation, and/or persistence in a range of soil types, including those with high clay and humic acid content, due to their known binding affinity for proteins.

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In addition, this study utilized field soil spiked with purified insecticidal protein. This approach is useful because dose responses can be easily quantified. However, the degradation and accumulation of Cry proteins found within decaying plant tissue may behave differently than proteins in artificially spiked soil. Thus, the relevance of these study results is unclear other than to show that degradation in soil does take place.

To account for the above concerns, it is recommended that additional studies should be conducted to evaluate insecticidal protein degradation, accumulation, and persistence in a variety of soil types, including those high in clay and humic acids, into which all non-harvested corn plant material is incorporated. Sampling should be conducted each year for three years in a field sown with continuous MIR604 corn. Soil should be monitored for a minimum of one growing season after harvest and monitoring should continue until mCry3A protein can no longer be detected. As noted in Table 13 below, the Agency has requested that the applicant submit this study as a condition of registration.

3. Effects on Soil Microorganisms

Numerous published studies indicate that exposure to Cry protein produced in *Bt* PIP crop plants does not adversely affect soil microorganisms. A minimal transient increase and shift in microbial populations was attributed to the presence of transgenic plant tissue in soil, however no adverse effects have been attributed to the Cry protein. A similar season-long field study with Cry3A potato also showed no adverse effects on soil microorganisms. There are several ongoing U.S. Department of Agriculture and EPA Office of Research and Development funded research projects addressing the effects of Cry protein crops on soil microbial flora. If adverse effects are seen from this research, the Agency will take appropriate action to mitigate potential risks.

4. Horizontal Transfer of Transgenes from *Bt* Crops to Soil Organisms

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

As a result of these findings, which suggest that hgt is at most an artificial event, and the fact that *Bt* toxins engineered into MIR604 were derived from soil-inhabiting bacteria, the EPA has concluded that there is a low probability of risk from hgt of transgenes found in mCry3A producing corn.

5. Gene Flow and Weediness Potential

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Movement of transgenes from crop plants into weeds is a significant concern, due to uncertainty regarding the effect that a new pest resistance gene may have on plant populations in the wild. Under FIFRA, the EPA has reviewed the potential for gene capture and expression of *Bt* endotoxins by wild or weedy relatives of corn, cotton, and potatoes in the U.S., its possessions and/or territories. To date, *Bt* plant-incorporated protectants have been registered for use in agronomic plant species that, for the most part, do not have a reasonable possibility of passing their traits to wild native plants. However, due to concern over the possibility that species related to corn (*Zea mays* ssp. *mays*), such as *Tripsacum* species and the teosintes, could be recipients of gene flow from genetically modified *Z. mays,* EPA conducted a thorough review of the scientific literature on what is known about the gene flow potential of *Z. mays* (*Bt* Reassessment Document, 2000). Conclusions gathered from this review process are as follows:

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

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

The October 2000 Scientific Advisory Panel agreed that the potential for gene transfer between corn and any receptive plants within the U.S., its possessions and territories was of limited probability and nearly risk free. As a result of these findings, the EPA has determined that there is no significant risk of gene capture and expression of mCry3A protein by wild or weedy relatives of corn in the U.S., its possessions or territories.

6. Impacts on Endangered Species (MRID 462656-01)

The primary route of exposure to mCry3A protein in corn is through ingestion of corn tissue. There are no reports of threatened or endangered species feeding on corn plants, therefore such species would not be exposed to corn tissue containing Cry protein. Since

mCry3A protein has not been shown to have toxicity effects on mammals, birds, plants, aquatic species, insects and other invertebrate species at the 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 mCry3A protein for coleopteran species, endangered species concerns are mainly restricted to the order Coleoptera. Examination of an overlay map showing the county level distribution of the 16 endangered/threatened 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 American burying beetle (*Nicrophorus americanus*). The American burying beetle is the largest carrion beetle in North America and is only found in limited areas of Rhode Island and portions of the Great Plains, including Arkansas and Georgia. Adults are nocturnal and feed on carrion and sometimes prey on other arthropods. Larvae feed exclusively on buried carrion provided by their parents. The American burying beetle's habitat is variable and often includes deciduous forest, grassland and agricultural areas. Considering that both larvae and adult insects feed exclusively on carrion, it appears that even if American burying beetles did occur in proximity to *Bt* corn fields, there would be little chance of exposure to *Bt* protein due to their feeding habits. After careful review of available data, the EPA determined that exposure of American burying beetle to harmful levels of MIR604 corn tissue is not expected. Likewise, a review of the preferred habitats of other coleopteran species listed as endangered by the U.S. Fish and Wildlife Service indicated that exposure to harmful levels of mCry3A protein would not take place. The main reasons for the lack of exposure are geographical and habitat limitations. These species are located in non-corn production areas and/or their habitat does not encompass agricultural areas.

Likewise, other insect species in the orders Diptera, Hemiptera, Lepidoptera, Odonata and Orthoptera that are listed as endangered/threatened species are found in dune, meadow/prairie or open forest habitats and are not closely associated with row crop production, often times due to the specificity of the habitat of their host plants. The reviewed toxicological data shows the relative insensitivity of a range of insects in non-Coleopteran orders to the mCry3A proteins, indicating that MIR604 maize hybrids are not likely to have detrimental effects on non-Coleopteran insects included on the endangered/threatened species list.

Further, several of the federally listed insect species are aquatic and consequently, are unlikely to come in contact with MIR604 maize plant material. Many of the endangered and threatened beetles occur in cave or aquatic habitats. Since movement into water bodies of soil containing mCry3A is expected to be negligible, pollen drift was considered the primary source of potential hazard to endangered aquatic Coleoptera. According to estimates based on published studies, if 100% of the pollen grains leaving a corn field were deposited in a 1 ha pond with 2 m depth and located ≥1 m from the edge

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of a corn field, <0.0001 µg mCry3A/mL of water would be expected. This is a few orders of magnitude below the toxic level to any insect.

Conclusion:
The reviewed non-target data confirm the expectation that MIR604 corn is not likely to jeopardize the continued existence of any endangered and/or threatened species listed by the US Fish and Wildlife Service, including mammals, birds or terrestrial and aquatic plants and invertebrate species. Therefore, no consultation with the USFWS is required under the Endangered Species Act.

B. Environmental Risk Assessment

The EPA uses a Maximum Hazard Dose Tiered system for biopesticide non-target wildlife hazard assessment. When no adverse effects at the maximum hazard dose are observed, the Agency concludes that there are no unreasonable adverse effects from the use of the pesticide.

At present, the Agency is aware of no identified significant adverse effects of mCry3A proteins on the abundance of non-target beneficial organisms in any population in the field environment, whether they are pest parasites, pest predators, or pollinators. Further, the EPA believes that cultivation of mCry3A corn may have fewer adverse impacts on non-target organisms than use of chemical pesticides for corn production, because under normal circumstances, mCry3A corn requires substantially fewer applications of chemical pesticides, compared to production of non-Bt corn. And fewer chemical insecticide applications generally result in increased populations of beneficial organisms that control secondary pests, such as aphids and leafhoppers, in corn fields. In addition, no adverse effect on endangered and threatened species listed by the US Fish and Wildlife Service is expected from the proposed MIR604 CRW resistant corn registration (see Section A.II.6 above). Further, the EPA has determined that there is no significant risk of gene capture and expression of mCry3A protein by wild or weedy relatives of corn in the U.S., its possessions, or territories (see Section A.II.5 above), available data do not indicate that Cry proteins have any measurable adverse effect on microbial populations in the soil (see Section A.II.3 above), nor has horizontal transfer of genes from transgenic plants to soil bacteria been demonstrated (see Section A.II.4 above). In conclusion, this risk assessment finds no hazard to the environment at the present time from cultivation of mCry3A protein expressing MIR604 corn for a time-limited registration.

C. Supplemental Studies Needed for Long Term MCry3A Non-Target Hazard Assessment

The Agency has sufficient information to believe that there is no risk from the proposed uses of mCry3A corn to non-target wildlife, aquatic, and soil organisms. However, in response to the August 2002 SAP recommendations, the Agency is requesting

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supplementary studies that will evaluate the persistence of mCry3A in the soil and the long range effects of cultivation of mCry3A on the invertebrate community structure in corn fields. This will facilitate identification of potential adverse effects which may result from long-term use of this product.

Table 13. Supplemental data requirements for Event MIR604 corn.

Testing Category	Type of Data
Ecosystem effects	Long range field studies should be conducted based on recommendations of the August, 2002 SAP.
Soil fate studies	Long range soil degradation field studies should be conducted. Studies should follow guidelines outlined by the August 2002 SAP, which are presented in summary form in the conclusion section of the soil fate review (see Section A.II.2 above).

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