

Modified Cry3A protein and the genetic material necessary for its production (via elements of pZM26) in event MIR604 corn SYN-IR604-8 (006509) Fact Sheet

Summary

EPA has conditionally registered Syngenta Seeds Inc.'s new active ingredient, modified Cry3A protein and the genetic material necessary for its production (via elements of pZM26) in event MIR604 corn SYN-IR604-8. The Agency has determined that the use of this pesticide is in the public interest and that it will not cause any unreasonable adverse effects on the environment during the time of conditional registration.

Results of efficacy trials conducted in 2002, 2003, and 2004 indicate that MIR604 corn provides effective control of key rootworm pests of field corn. MIR604 corn has unique biochemical properties which may benefit insect resistance management for this and other CRW-protected corn products. MIR604 contains the third CRW-active Bt corn protein on the market. The availability of multiple CRW-protected corn products will increase grower choice and price competition, resulting in lower seed prices for growers and higher adoption rates. Registration of MIR604 corn is expected to result in further reduction of chemical insecticide use by growers. This is of special importance since many pesticides registered for CRW-control are highly toxic to humans and the environment, while mCry3A-expressing corn poses no foreseeable human health or environmental risks.

The new corn plant-incorporated protectant, Event MIR604 corn, produces its own insecticide within the corn plant. This protectant, mCry3A protein, is derived from *Bacillus thuringiensis* (Bt), a naturally occurring soil bacterium. The mCry3A protein used in this product controls corn rootworm, a highly destructive pest responsible for the single largest use of conventional insecticides in the United States.

In order to reduce the possibility of corn rootworm developing resistance to Bt, EPA is requiring Syngenta Seeds, Inc. to ensure that 20 percent of the planted acreage of this product be set aside in which non-CRW-protected corn will be grown to serve as a "refuge." These refuge areas will support populations of corn rootworm not exposed to the CRW-protected corn. The insect populations in the refuges will help prevent resistance development when they cross-breed with insects in the CRW-protected fields. This resistance management strategy was developed as a condition of the registration, and EPA will require routine monitoring and documentation that these measures are followed. The submitted insect resistance management data support a registration until 2010.

A tolerance exemption under 40 CFR Part 174.456 is in place for *Bacillus thuringiensis* modified Cry3A protein and the genetic material necessary for its production in corn.

I. Target Pests/ Application Sites & Methods

- **Pesticide Name:** Modified Cry3A protein and the genetic material necessary for its production (via elements of pZM26) in event MIR604 corn SYN-IR604-8
- **Date Registered:** October 3, 2006
- **Registration Numbers:** 67979-5
- **Trade and Other Names:** AgrisureT RW Rootworm-Protected Corn; Event MIR604 Corn
- **OPP Chemical Code:** 006509
- **Basic Manufacturers:**
Syngenta Seeds, Inc. - Field Crops - NAFTA
P.O. Box 12257, 3054 East Cornwallis Road
Research Triangle Park, NC 27709-2257
- **Type of Pesticide:** Plant-Incorporated Protectant
- **Uses:** Field Corn
- **Target Pest(s):** Corn Rootworm

II. Science Assessment

A. Product Characterization

The modified Cry3A (mCry3A) *Bacillus thuringiensis* (Bt) insect control protein is produced in transgenic corn plants derived from transformation Event MIR604 and has activity against certain beetles. A cry3A gene from Bt subsp. *tenebrionis* was recreated synthetically to optimize for expression in corn. Additional changes in this corn-optimized gene were made, such that the encoded mCry3A protein has enhanced activity against larvae of the western corn rootworm and northern corn rootworm.

Protein characterization data demonstrate that the plant-produced protein is of sufficient biological activity to those of the two modified Cry3A protein variants produced in the recombinant *E. coli* test system (designated as test material MCRY3A-0102) for the purposes of human health and ecological effects risk assessments. Although the MCRY3A-0102 test material was not as active towards target pests as the plant-produced modified Cry3A protein, the doses in submitted studies were much higher than would occur via the modified corn.

B. Human Health Assessment

There is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the mCry3A protein and the genetic material necessary for its production.

An acute oral toxicity study was submitted for the mCry3A protein and supports the prediction that the mCry3A protein would be non-toxic to humans. Male and female mice (5 of each) were dosed with 2,377 milligrams/kilograms bodyweight (mg/kg bwt) of mCry3A protein. With the exception of one female in the test group that was euthanized on day 2 (due to adverse clinical signs consistent with a dosing injury), all other mice survived the study, gained weight, had no test material-related clinical signs, and had no test material-related findings at necropsy.

Amino acid sequence comparisons showed no similarity between the mCry3A protein to known toxic proteins available in public protein data bases.

Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by acid and proteases; may be glycosylated; and present at high concentrations in the food. Data have been submitted that demonstrate that the mCry3A protein is rapidly degraded by gastric fluid *in vitro*. In a solution of simulated gastric fluid, 1 mg/mL mCry3A test protein mixed with simulated gastric fluid (pH 1.2, containing 2 mg/mL NaCl, 14 mL 6 N HCl, and 2.7 mg/mL pepsin) resulting in 10 pepsin activity units/ mg test protein (complies with 2000 US Pharmacopoeia recommendations), complete degradation of detectable mCry3A protein occurred within 2 minutes. A comparison of amino acid sequences of known allergens uncovered no evidence of any homology with mCry3A, even at the level of 8 contiguous amino acid residues. Further data demonstrate that mCry3A is not glycosylated and is present in low levels in corn tissue.

C. Environmental Assessment

The Agency is aware of no identified significant adverse effects of mCry3A protein 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. In addition, 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. 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. 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.

D. Insect Resistance Management

The proposed IRM strategy and data to support it are "acceptable" except that the in-field strip refuge must be at least 4 rows wide (=6 rows wide preferred) based on recent larval movement data. If resistance is recessive, then the proposed IRM plan using a 20% structured refuge will be adequate to delay resistance for at least 15 years given the assumptions of Syngenta's model. If MIR604 maize is planted in areas with observable rotation-resistance in WCRW, then planting transgenic corn only in rotated maize fields is a good IRM strategy that will delay the evolution of resistance by at least 15 years regardless of gene expression.

E. Benefits

Registration of modified Cry3A protein and the genetic material necessary for its production (via elements of pZM26) in event MIR604 corn SYN-IR604-8 is in the public interest because:

1. Results of efficacy trials conducted in 2002, 2003, and 2004 indicate that MIR604 corn provides effective control of key rootworm pests of field corn.
2. MIR604 corn has unique biochemical properties which may benefit insect resistance management for this and other CRW-protected corn products.
3. MIR604 corn is registered, it will be the third CRW-protected *Bt* corn product on the market. The availability of multiple CRW-protected corn products will increase grower choice and price competition, resulting in lower seed prices for growers and higher adoption rates.
4. Registration of MIR604 corn is expected to result in further reduction of chemical insecticide use by growers. This is of special importance since many pesticides registered for CRW-control are highly toxic to humans and the environment, while mCry3A-expressing corn poses no foreseeable human health or environmental risks.

III. Terms and Conditions of the Registration

. Specific Terms and Conditions

1. The subject registration will automatically expire on midnight September 30, 2010.
2. The subject registration will be limited to mCry3A corn, modified Cry3A protein and the genetic material necessary for its production (via elements of pZM26) in Event MIR604 corn SYN-IR604-8.

3. Submit/cite all data required for registration of your product under FIFRA § 3(c)(5) when the Agency requires registrants of similar products to submit such data.
4. Provide to the EPA laboratory (Ft. Meade, MD) methodology and/or reagents necessary for validation of a mCry3A analytical method within 6 months from the date that the Agency requests them.
5. Submit field degradation studies evaluating accumulation and persistence of mCry3A in several different soils in various strata. Representative fields must have been planted with mCry3A corn and include both conventional tillage and no-till samples and be harvested under typical agronomic conditions. Sampling must continue until the limit of detection is reached. Studies should include soils with high levels of a variety of clays. Both ELISA and insect bioassays need to be conducted and compared to determine if mCry3A is accumulating or persisting in soil samples. A protocol is due within 90 days of the date of registration. Should the registration expiration date be extended, a final report regarding data from fields that have had three continuous years of cultivation of Event MIR604 corn is due by January 31, 2011.
6. Three (3) year full-scale field or semi-field studies for evaluation of mCry3A Event MIR604 corn exposure on non-target invertebrates must be conducted. Full-scale field experiments must be appropriately designed to provide a measure of ecological impacts (larger fields, more replicates, more samples per plot based on recommendations of the August, 2002 SAP and subsequent relevant research on appropriate study design). A protocol is due within 90 days of the date of registration. Should the registration expiration date be extended, a final report is due January 31, 2011.
7. Submit the following data to augment the mCry3A amino acid sequence analysis to known toxins and allergens within six months of the date of registration: specification of which version of NCBI database was utilized; descriptions of parameters utilized; and dates accessed for the BLAST search.
8. Submit the following insect resistant management data by January 31, 2010.
 - a. Initiate establishment of CRW strains that are resistant to mCry3A and investigate the nature, inheritance, and fitness costs of specific mechanisms of resistance to the mCry3A protein expressed in MIR604 maize.
 - b. Study the behavioral deterrence (avoidance) mechanism further and submit appropriate results.
 - c. Continue studies on the biological impact of adults surviving on MIR604 maize and submit these results.
9. You must do the following Insect Resistance Management Program.
 - a. **Refuge Requirements**

These refuge requirements do not apply to seed increase/propagation of inbred and hybrid seed corn.

1. Grower agreements (also known as stewardship agreements) will specify that growers must adhere to the refuge requirements as described in the grower guide/product use guide and/or in supplements to the grower guide/product use guide.
2. Specifically, growers must plant a structured refuge of at least 20% non-corn rootworm protected Bt corn that may be treated with insecticides as needed to control corn rootworm larvae. Growers will not be permitted to apply CRW labeled insecticides to the refuge for control of insect pests while adult corn rootworm are present unless the mCry3A field is treated in a similar manner.
3. Refuge planting options include: refuge acres should be planted as blocks adjacent to mCry3A MIR604 corn fields, perimeter strips, or as in-field strips.
4. External refuges must be planted adjacent (e.g., across the road) to mCry3A MIR604 fields.
5. When planting the refuge in strips across the field, refuges must be at least 4 rows wide, preferably 6 consecutive rows wide.
6. Insecticide treatments for control of corn rootworm larvae may be applied. Instructions to growers will specify that insecticides labeled for control of corn rootworm adults cannot be applied while adults are present in the refuge unless the mCry3A field is treated in a similar manner.
7. If the refuge is planted in a field that is in a crop rotation system, then MIR604 must also be planted in a field that is in a crop rotation system.
8. If the refuge is planted on continuous corn, then the MIR604 field may be planted on either continuous or in a field that is in a crop rotation system.

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B. Grower Agreements

1. Persons purchasing the Bt corn product must sign a grower agreement. The term "grower agreement" refers to any grower purchase contract, license agreement, or similar legal document.
2. The grower agreement and/or specific stewardship documents referenced in the grower agreement must clearly set forth the terms of the current IRM program. By signing the grower agreement, a grower must be contractually bound to comply with the requirements of the IRM program.
3. The registrant must develop a system (equivalent to what is already approved for Bt11 field corn, EPA Reg. No. 67979-1) which is reasonably likely to assure that persons purchasing the Bt corn product will affirm annually that they are contractually bound to comply with the requirements of the IRM program. The proposed system will be submitted to EPA within 90 days from the date of registration.
4. The registrant must use grower agreements and submit to EPA within 90 days from the date of registration a copy of that agreement and any specific stewardship documents referenced in the grower agreement. If Syngenta wishes to change any part of the grower agreement or any specific stewardship documents referenced in the grower agreement that would affect either the content of the IRM program or the legal enforceability of the provisions of the agreement relating to the IRM program, thirty days prior to implementing a proposed change, the registrant must submit to EPA the text of such changes to ensure that it is consistent with the terms and conditions of the registration.
5. The registrant must establish a system (equivalent to what is already approved for Bt11, EPA Reg. No. 67979-1) which is reasonably likely to assure that persons purchasing the Bt corn sign grower agreement(s), and must provide within 90 days from the date of the registration a written description of that system.

6. The registrant shall maintain records of all Bt corn grower agreements for a period of three years from December 31st of the year in which the agreement was signed.
7. Beginning on January 31, 2008 and annually thereafter, the registrant shall provide EPA with a report showing the number of units of its Bt MIR604 corn seeds sold or shipped and not returned, and the number of such units that were sold to persons who have signed grower agreements. The report shall cover the time frame of the twelve-month period covering the prior August through July.
8. Beginning in January 31, 2008, and annually thereafter, the registrant shall submit annual reports on units sold by State (units sold by county level will be made available to the Agency upon request), IRM grower agreement results, and the compliance assurance program, including the education program on or before January 31st each year.
9. The registrant must allow a review of the grower agreements and grower agreement records by EPA or by a State pesticide regulatory agency if the State agency can demonstrate that confidential business information, including names, personal information, and grower license number, will be protected.

C. IRM Education and IRM Compliance Monitoring Programs

1. Syngenta must design and implement a comprehensive, ongoing IRM education program designed to convey to Bt MIR604 corn users the importance of complying with the IRM program. The program shall include information encouraging Bt MIR604 corn users to pursue optional elements of the IRM program relating to refuge configuration and proximity to Bt MIR604 corn fields. The education program shall involve the use of multiple media, e.g. face-to-face meetings, mailing written materials, EPA reviewed language on IRM requirements on the bag or bag tag, and electronic communications such as by Internet, radio, or television commercials. Copies of the materials will be provided to EPA for its records. The program shall involve at least one written communication annually to each Bt MIR604 corn user separate from the grower technical guide. The communication shall inform the user of the current IRM requirements. Syngenta shall coordinate its education programs with educational efforts of other registrants and other organizations, such as the National Corn Growers Association and state extension programs.
2. Annually, the registrant shall revise, and expand as necessary, its education program to take into account the information collected through the compliance survey required under paragraph 6] and from other sources. The changes shall address aspects of grower compliance that are not sufficiently high.
3. On January 31, 2008, the registrant must provide a report to EPA summarizing the activities carried out under the education program for the prior year. Annually thereafter, the registrant must provide EPA any substantive changes to its grower education activities as part of the overall IRM compliance assurance program report. The required features of the compliance assurance program are described in paragraphs 4]-15] below.
4. The registrant must design and implement an ongoing IRM compliance assurance program designed to evaluate the extent to which growers purchasing its MIR604 Bt corn product are complying with the IRM program and that takes such actions as are reasonably needed to assure

that growers who have not complied with the program either do so in the future or lose their access to the MIR604 Bt corn product. The registrant shall coordinate with other Bt corn registrants in designing and implementing its compliance assurance program and integrate the mCry3A CAP with the CAP already approved for Bt11, EPA Registration Number 67979-1. The registrant must prepare and submit within 90 days of the date of registration a written description of their compliance assurance program. Other required features of the program are described in paragraphs 5] - 15] below.

5. The registrant must establish and publicize a "phased compliance approach," i.e., a guidance document that indicates how the registrant will address instances of non-compliance with the terms of the IRM program and general criteria for choosing among options for responding to any non-compliant growers. The options shall include withdrawal of the right to purchase MIR604 Bt corn for an individual grower or for all growers in a specific region. An individual grower found to be significantly out of compliance two years in a row would be denied sales of the product the next year. Similarly, seed dealers who are not fulfilling their obligations to inform/educate growers of their IRM obligations will lose their opportunity to sell MIR604 Bt corn.
6. The IRM compliance assurance program shall include an annual survey conducted by an independent third party of a statistically representative sample of growers of Bt corn products who plant the vast majority of all corn in the U.S. and in areas in which the selection intensity is greatest. The survey shall consider only those growers who plant 200 or more acres of corn in the Corn-Belt and who plant 100 or more acres of corn in corn-cotton areas. The survey shall measure the degree of compliance with the IRM program by growers in different regions of the country and consider the potential impact of non-response. The sample size and geographical resolution may be adjusted annually, based upon input from the independent marketing research firm and academic scientists, to allow analysis of compliance behavior within regions or between regions. The sample size must provide a reasonable sensitivity for comparing results across the U.S.
7. The survey shall be designed to provide an understanding of any difficulties growers encounter in implementing IRM requirements. An analysis of the survey results must include the reasons, extent, and potential biological significance of any implementation deviations.
8. The survey shall be designed to obtain grower feedback on the usefulness of specific educational tools and initiatives.
9. The registrant shall provide a final written summary of the results of the prior year's survey (together with a description of the regions, the methodology used, and the supporting data) to EPA by January 31 of each year, beginning with 2008. The registrant shall confer with other Bt corn registrants and EPA on the design and content of the survey prior to its implementation.
10. Annually, the registrant shall revise, and expand as necessary, its compliance assurance program to take into account the information collected through the compliance survey required under paragraphs 6] through 8] and from other sources. The changes shall address aspects of grower compliance that are not sufficiently high. The registrant must

confer with the Agency prior to adopting any changes to a previously approved CAP.

11. The registrant shall conduct an annual on-farm assessment program. The registrant shall train its representatives who make on-farm visits with growers of their Bt corn products to perform assessments of compliance with IRM requirements. There is no minimum corn acreage size for this program. Therefore, growers will be selected for this program from across all farm sizes. In the event that any of these visits result in the identification of a grower who is not in compliance with the IRM program, the registrant shall take appropriate action, consistent with its "phased compliance approach," to promote compliance.
12. The registrant shall carry out a program for investigating legitimate "tips and complaints" that its growers are not in compliance with the IRM program. Whenever an investigation results in the identification of a grower who is not in compliance with the IRM program, the registrant shall take appropriate action, consistent with its "phased compliance approach."
13. If a grower, who purchases MIR604 Bt corn for planting, was specifically identified as not being in compliance during the previous year, the registrant shall visit with the grower and evaluate whether that the grower is in compliance with the IRM program for the current year.
14. Beginning January 31, 2008 and annually thereafter, Syngenta shall provide a report to EPA summarizing the activities carried out under their compliance assurance program for the prior year and the plans for the compliance assurance program during the current year. The report will include information regarding grower interactions (including, but not limited to, on-farm visits, verified tips and complaints, grower meetings and letters), the extent of non-compliance, corrective measures to address the non-compliance, and any follow-up actions taken.
15. The registrant and the seed corn dealers for the registrant must allow a review of the compliance records by EPA or by a State pesticide regulatory agency if the State agency can demonstrate that confidential business information, including the names, personal information, and grower license number of the growers will be protected.

D. Insect Resistance Monitoring

The Agency is imposing the following conditions for this product

The registrant must monitor for mCry3A resistance and/or trends in increased tolerance for corn rootworm. Sampling should be focused in those areas in which there is the highest risk of resistance development.

1. The registrant must provide EPA its resistance monitoring plan for approval. A revised monitoring plan must be submitted to the Agency with 3 months of the date of registration consisting of a description of the steps to be taken to establish corn rootworm baseline sensitivity and damage guidelines. A detailed resistance monitoring plan must be submitted to the Agency for review by January 31, 2008. This plan must include: baseline sensitivity data, sampling (number of locations, samples per locations), sampling methodology and life-stage sampled, bioassay methodology,

standardization procedures (including QA/QC provisions), detection technique and sensitivity, the statistical analysis of the probability of detecting resistance, and an interim description of rootworm damage guidelines.

2. The registrant must develop and validate an appropriate discriminating or diagnostic dose assay by January 31, 2010.
3. You must finalize rootworm damage guidelines and submit these to BPPD by January 31, 2010.
4. The registrant must follow-up on grower, extension specialist or consultant reports of unexpected damage or control failures for corn rootworm.
5. The registrant must provide EPA with an annual resistance monitoring report by August 31st of each year beginning with 2008, reporting on populations collected the previous year.

E. Remedial Action Plans

The following program summary describes, in order of events, the steps that must be taken to implement a remedial action plan if resistance to target pests is confirmed (this general process has been implemented for other lepidopteron and CRW Bt corn products).

1. Definition of Suspected Resistance:

Resistance will be suspected if investigations of unexpected damage reports show that:

- a. implicated maize plant roots were expressing the mCry3A protein at the expected level;
- b. alternative causes of damage or lodging, such as non-target pest insect species, weather, physical damage, larval movement from alternate hosts, planting errors, and other reasonable causes for the observations, have been ruled out;
- c. the level of damage exceeds guidelines for expected damage.

If resistance is "suspected", the registrants will instruct affected growers to use alternate pest control measures such as adulticide treatment, crop rotation the following year, or use of soil or seed insecticides the following year. These measures are intended to reduce the possibility of potentially resistant insects contributing to the following year's pest population.

2. Confirmation of Resistance:

Resistance will be confirmed if all of the following criteria are met by progeny from the target pest species sampled from the area of "suspected resistance":

- a. the proportion of larvae that can feed and survive on mCry3A roots from neonate to adult is significantly higher than the baseline proportion (currently being established);
- b. the LC50 of the test population exceeds the upper limit of the 95% confidence interval for the LC50 of a standard unselected population and/or survival in the diagnostic assay is significantly greater than that of a standard unselected population, as established by the ongoing baseline monitoring program;
- c. the ability to survive is heritable;
- d. mCry3A plant assays determine that damage caused by surviving insects would exceed economic thresholds;
- e. the identified frequency of field resistance could lead to widespread product failure if subsequent collections in the affected field area(s) demonstrated similar bioassay results.

3. **Response to Confirmed Resistance:**

When resistance is "confirmed", the following steps will be taken:

- a. EPA will receive notification within 30 days of resistance confirmation;
- b. affected customers and extension agents will be notified about confirmed resistance;
- c. affected customers and extension agents will be encouraged to employ alternative CRW control measures;
- d. sale and distribution of mCry3A maize in the affected area will cease immediately
- e. a long-term resistance management action plan will be devised according to the characteristics of the resistance event and local agronomic needs. [The details of such a plan should be approved by EPA and all appropriate stakeholders.]

F. Annual Reporting Requirements

1. Annual Sales: reported and summed by state (county level data available by request), January 31st each year;
2. Grower Agreement: number of units of Bt corn seeds shipped or sold and not returned, and the number of such units that were sold to persons who have signed grower agreements, January 31st each year;
3. Grower Education: substantive changes to education program completed previous year, January 31st each year;

4. Compliance Assurance Plan: Compliance Assurance Program activities and results, January 31st each year;
5. Compliance: to include annual survey results and plans for the next year; full report January 31st each year;
6. Insect Resistance Monitoring Results: results of monitoring and investigations of damage reports, August 31st each year, beginning in 2008.

IV. Additional Contact Information

[Ombudsman, Biopesticides and Pollution Prevention Division](#) (7511P)
Office of Pesticide Programs
Environmental Protection Agency
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Washington, D.C. 20460