

**ENVIRONMENTAL PROTECTION AGENCY**

[OPP-2004-0226; FRL-7369-8]

**Modified Cry3A Protein (mCry3A) and the Genetic Material Necessary for its Production in Corn; Notice of Filing a Pesticide Petition to Establish a Temporary Tolerance for a Certain Pesticide Chemical in or on Food****AGENCY:** Environmental Protection Agency (EPA).**ACTION:** Notice.

**SUMMARY:** This notice announces the initial filing of a pesticide petition proposing the establishment of regulations for residues of a certain pesticide chemical in or on various food commodities.

**DATES:** Comments, identified by docket identification (ID) number OPP-2004-0226, must be received on or before September 30, 2004.

**ADDRESSES:** Comments may be submitted electronically, by mail, or through hand delivery/courier. Follow the detailed instructions as provided in Unit I. of the **SUPPLEMENTARY INFORMATION**.

**FOR FURTHER INFORMATION CONTACT:** Mike Mendelsohn, Biopesticides and Pollution Prevention Division (7511C), Office of Pesticide Programs, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460-0001; telephone number: (703) 308-8715; e-mail address: [mendelsohn.mike@epa.gov](mailto:mendelsohn.mike@epa.gov).

**SUPPLEMENTARY INFORMATION:****I. General Information***A. Does this Action Apply to Me?*

You may be potentially affected by this action if you are an agricultural producer, food manufacturer, or pesticide manufacturer. Potentially affected entities may include, but are not limited to:

- Crop production (NAICS 111)
- Animal production (NAICS 112)
- Food manufacturing (NAICS 311)
- Pesticide manufacturing (NAICS 32532)

This listing is not intended to be exhaustive, but rather provides a guide for readers regarding entities likely to be affected by this action. Other types of entities not listed in this unit could also be affected. The North American Industrial Classification System (NAICS) codes have been provided to assist you and others in determining whether this action might apply to certain entities. If you have any questions regarding the applicability of

this action to a particular entity, consult the person listed under **FOR FURTHER INFORMATION CONTACT**.

*B. How Can I Get Copies of this Document and Other Related Information?*

1. *Docket.* EPA has established an official public docket for this action under docket ID number OPP-2004-0226. The official public docket consists of the documents specifically referenced in this action, any public comments received, and other information related to this action. Although a part of the official docket, the public docket does not include Confidential Business Information (CBI) or other information whose disclosure is restricted by statute. The official public docket is the collection of materials that is available for public viewing at the Public Information and Records Integrity Branch (PIRIB), Rm. 119, Crystal Mall #2, 1801 S. Bell St., Arlington, VA. This docket facility is open from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The docket telephone number is (703) 305-5805.

2. *Electronic access.* You may access this **Federal Register** document electronically through the EPA Internet under the "**Federal Register**" listings at <http://www.epa.gov/fedrgstr/>.

An electronic version of the public docket is available through EPA's electronic public docket and comment system, EPA Dockets. You may use EPA Dockets at <http://www.epa.gov/edocket/> to submit or view public comments, access the index listing of the contents of the official public docket, and to access those documents in the public docket that are available electronically. Although not all docket materials may be available electronically, you may still access any of the publicly available docket materials through the docket facility identified in Unit I.B.1. Once in the system, select "search," then key in the appropriate docket ID number.

Certain types of information will not be placed in EPA's Dockets. Information claimed as CBI and other information whose disclosure is restricted by statute, which is not included in the official public docket, will not be available for public viewing in EPA's electronic public docket. EPA's policy is that copyrighted material will not be placed in EPA's electronic public docket but will be available only in printed, paper form in the official public docket. To the extent feasible, publicly available docket materials will be made available in EPA's electronic public docket. When a document is selected from the index list in EPA Dockets, the system will identify whether the document is

available for viewing in EPA's electronic public docket. Although not all docket materials may be available electronically, you may still access any of the publicly available docket materials through the docket facility identified in Unit I.B.1. EPA intends to work towards providing electronic access to all of the publicly available docket materials through EPA's electronic public docket.

For public commenters, it is important to note that EPA's policy is that public comments, whether submitted electronically or in paper, will be made available for public viewing in EPA's electronic public docket as EPA receives them and without change, unless the comment contains copyrighted material, CBI, or other information whose disclosure is restricted by statute. When EPA identifies a comment containing copyrighted material, EPA will provide a reference to that material in the version of the comment that is placed in EPA's electronic public docket. The entire printed comment, including the copyrighted material, will be available in the public docket.

Public comments submitted on computer disks that are mailed or delivered to the docket will be transferred to EPA's electronic public docket. Public comments that are mailed or delivered to the docket will be scanned and placed in EPA's electronic public docket. Where practical, physical objects will be photographed, and the photograph will be placed in EPA's electronic public docket along with a brief description written by the docket staff.

*C. How and to Whom Do I Submit Comments?*

You may submit comments electronically, by mail, or through hand delivery/courier. To ensure proper receipt by EPA, identify the appropriate docket ID number in the subject line on the first page of your comment. Please ensure that your comments are submitted within the specified comment period. Comments received after the close of the comment period will be marked "late." EPA is not required to consider these late comments. If you wish to submit CBI or information that is otherwise protected by statute, please follow the instructions in Unit I.D. Do not use EPA Dockets or e-mail to submit CBI or information protected by statute.

1. *Electronically.* If you submit an electronic comment as prescribed in this unit, EPA recommends that you include your name, mailing address, and an e-mail address or other contact information in the body of your

comment. Also include this contact information on the outside of any disk or CD ROM you submit, and in any cover letter accompanying the disk or CD ROM. This ensures that you can be identified as the submitter of the comment and allows EPA to contact you in case EPA cannot read your comment due to technical difficulties or needs further information on the substance of your comment. EPA's policy is that EPA will not edit your comment, and any identifying or contact information provided in the body of a comment will be included as part of the comment that is placed in the official public docket, and made available in EPA's electronic public docket. If EPA cannot read your comment due to technical difficulties and cannot contact you for clarification, EPA may not be able to consider your comment.

i. *EPA Dockets.* Your use of EPA's electronic public docket to submit comments to EPA electronically is EPA's preferred method for receiving comments. Go directly to EPA Dockets at <http://www.epa.gov/edocket/>, and follow the online instructions for submitting comments. Once in the system, select "search," and then key in docket ID number OPP-2004-0226. The system is an "anonymous access" system, which means EPA will not know your identity, e-mail address, or other contact information unless you provide it in the body of your comment.

ii. *E-mail.* Comments may be sent by e-mail to [opp-docket@epa.gov](mailto:opp-docket@epa.gov), Attention: Docket ID Number OPP-2004-0226. In contrast to EPA's electronic public docket, EPA's e-mail system is not an "anonymous access" system. If you send an e-mail comment directly to the docket without going through EPA's electronic public docket, EPA's e-mail system automatically captures your e-mail address. E-mail addresses that are automatically captured by EPA's e-mail system are included as part of the comment that is placed in the official public docket, and made available in EPA's electronic public docket.

iii. *Disk or CD ROM.* You may submit comments on a disk or CD ROM that you mail to the mailing address identified in Unit I.C.2. These electronic submissions will be accepted in WordPerfect or ASCII file format. Avoid the use of special characters and any form of encryption.

2. *By mail.* Send your comments to: Public Information and Records Integrity Branch (PIRIB) (7502C), Office of Pesticide Programs (OPP), Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington,

DC 20460-0001, Attention: Docket ID Number OPP-2004-0226.

3. *By hand delivery or courier.* Deliver your comments to: Public Information and Records Integrity Branch (PIRIB), Office of Pesticide Programs (OPP), Environmental Protection Agency, Rm. 119, Crystal Mall #2, 1801 S. Bell St., Arlington, VA, Attention: Docket ID Number OPP-2004-0226. Such deliveries are only accepted during the docket's normal hours of operation as identified in Unit I.B.1.

#### *D. How Should I Submit CBI to the Agency?*

Do not submit information that you consider to be CBI electronically through EPA's electronic public docket or by e-mail. You may claim information that you submit to EPA as CBI by marking any part or all of that information as CBI (if you submit CBI on disk or CD ROM, mark the outside of the disk or CD ROM as CBI and then identify electronically within the disk or CD ROM the specific information that is CBI). Information so marked will not be disclosed except in accordance with procedures set forth in 40 CFR part 2.

In addition to one complete version of the comment that includes any information claimed as CBI, a copy of the comment that does not contain the information claimed as CBI must be submitted for inclusion in the public docket and EPA's electronic public docket. If you submit the copy that does not contain CBI on disk or CD ROM, mark the outside of the disk or CD ROM clearly that it does not contain CBI. Information not marked as CBI will be included in the public docket and EPA's electronic public docket without prior notice. If you have any questions about CBI or the procedures for claiming CBI, please consult the person listed under **FOR FURTHER INFORMATION CONTACT.**

#### *E. What Should I Consider as I Prepare My Comments for EPA?*

You may find the following suggestions helpful for preparing your comments:

1. Explain your views as clearly as possible.
2. Describe any assumptions that you used.
3. Provide copies of any technical information and/or data you used that support your views.
4. If you estimate potential burden or costs, explain how you arrived at the estimate that you provide.
5. Provide specific examples to illustrate your concerns.
6. Make sure to submit your comments by the deadline in this notice.

7. To ensure proper receipt by EPA, be sure to identify the docket ID number assigned to this action in the subject line on the first page of your response. You may also provide the name, date, and **Federal Register** citation.

## **II. What Action is the Agency Taking?**

EPA has received a pesticide petition as follows proposing the establishment and/or amendment of regulations for residues of a certain pesticide chemical in or on various food commodities under section 408 of the Federal Food, Drug, and Cosmetic Act (FFDCA), 21 U.S.C. 346a. EPA has determined that this pesticide petition contains data or information regarding the elements set forth in FFDCA section 408(d)(2); however, EPA has not fully evaluated the sufficiency of the submitted data at this time or whether the data support granting of the pesticide petition. Additional data may be needed before EPA rules on the pesticide petition.

### **List of Subjects**

Environmental protection, Agricultural commodities, Feed additives, Food additives, Pesticides and pests, Reporting and recordkeeping requirements.

Dated: August 23, 2004.

**Janet L. Andersen,**

*Director, Biopesticides and Pollution Prevention Division, Office of Pesticide Programs.*

### **Summary of Petition**

The petitioner's summary of the pesticide petition (PP) is printed below as required by FFDCA section 408(d)(3). The summary of the petition was prepared by the petitioner and represents the view of the petitioner. The petition summary announces the availability of a description of the analytical methods available to EPA for the detection and measurement of the pesticide chemical residues or an explanation of why no such method is needed.

### **Syngenta Seeds, Inc.**

*PP 4G6808*

EPA has received a pesticide petition (4G6808) from Syngenta Seeds, Inc., P.O. Box 12257, 3054 Cornwallis Road, Research Triangle Park, NC 27709-2257, proposing pursuant to section 408(d) of the FFDCA, 21 U.S.C. 346(d), to amend 40 CFR part 174 to establish an exemption from the requirement of a tolerance for the plant-pesticide modified Cry3A protein (mCry3A) and the genetic material necessary for its production in or on all corn.

Pursuant to section 408(d)(2)(A)(i) of the FFDCFA, as amended, Syngenta Seeds, Inc., has submitted the following summary of information, data, and arguments in support of their pesticide petition. This summary was prepared by Syngenta Seeds, Inc., and EPA has not fully evaluated the merits of the pesticide petition. The summary may have been edited by EPA if the terminology used was unclear, the summary contained extraneous material, or the summary unintentionally made the reader conclude that the findings reflected EPA's position and not the position of the petitioner.

#### A. Product Name and Proposed Use Practices

A modified Cry3A *Bacillus thuringiensis* insect control protein and the genetic material necessary for its production in Event MIR604-derived corn is proposed for use as a plant-incorporated protectant active ingredient. Production of the mCry3A protein within corn plants confers resistance to damage caused by the western corn rootworm and northern corn rootworm, which are major corn pests in the United States. A temporary exemption from tolerances is being requested in conjunction with a proposed Experimental Use Permit to allow large-scale field testing of field corn plants derived from Syngenta Seeds' transformation event MIR604.

#### B. Product Identity/Chemistry

1. *Identity of the pesticide and corresponding residues.* A modified Cry3A (mCry3A) *Bacillus thuringiensis* insect control protein is produced in transgenic corn plants derived from transformation Event MIR604. A *cry3A* gene from *Bacillus thuringiensis* subspecies (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 (*diabrotica virgifera virgifera*) and northern corn rootworm (*diabrotica longicornis barberi*). Event MIR604-derived corn plants express the synthetic modified *cry3A* gene, introduced via transformation vector pZM26, and display resistance to these pests. The native Cry3A protein of *Bacillus thuringiensis* subsp. *tenebrionis* is a carbon absorber (ca) 73 kDa polypeptide of 644 amino acids. By comparison, the mCry3A protein expressed in Event MIR604 corn is a ca 67 kDa polypeptide of 598 amino acids. Its amino acid sequence corresponds to that of the native Cry3A protein, except that (1) its

N-terminus corresponds to methionine-48 of the native protein and (2) a cathepsin G protease recognition site has been introduced into the protein, conferring enhanced activity toward western and northern corn rootworms. Residues of the mCry3A protein, and/or breakdown products thereof, are present in corn grain and other tissues of Event MIR604-derived plants.

2. *Magnitude of residue at the time of harvest and method used to determine the residue.* A determination of the magnitude of residue at harvest is not required for residues exempt from tolerances. However, the petitioner has provided data on the quantity of mCry3A protein measured in various plant parts. Average mCry3A levels in grain from Event MIR604-derived hybrid field corn plants were less than 1 part per million (ppm) on a dry-weight or fresh-weight basis, as measured by enzyme linked immunosorbent assay (ELISA). Average mCry3A levels measured in chopped whole Event MIR604-derived hybrid corn plants were less than or equal to ca. 20 ppm on a dry-weight basis and less than or equal to ca. 8 ppm on a fresh-weight basis.

3. *A statement of why an analytical method for detecting and measuring the levels of the pesticide residue are not needed.* An analytical method is not required because this petition requests an exemption from tolerances. However, the petitioner has submitted an analytical method for detection of the mCry3A protein by ELISA.

#### C. Mammalian Toxicological Profile

Syngenta Seeds is providing the results of a mammalian toxicology study, *in vitro* digestibility study, heat stability study and bioinformatics evaluations conducted on the mCry3A protein. These studies, summarized herein, demonstrate the lack of toxicity of the mCry3A protein following acute oral high-dose exposure to mice, rapid degradation of mCry3A upon exposure to simulated mammalian gastric fluid, instability of the mCry3A protein upon heating, and the lack of significant amino acid sequence homology of the mCry3A protein to proteins known to be mammalian toxins or human allergens.

When proteins are toxic, they are known to act *via* acute mechanisms and at very low doses, Sjoblad, R.D., J.T. McClintock and R. Engler (1992) *Toxicological Considerations for Protein Components of Biological Pesticide Products. Regulatory Toxicology and Pharmacology*. 15: 3–9. Therefore, when a protein demonstrates no acute oral toxicity in high-dose testing using a standard laboratory mammalian test

species, this supports the determination that the protein will be non-toxic to humans and other mammals, and will not present a hazard under any realistic exposure scenario, including long-term exposures.

Because it is not feasible to extract sufficient mCry3A protein from transformed plants for toxicology studies, mCry3A protein was produced in recombinant *E. coli* by over-expressing the same modified *cry3A* gene that was introduced into Event MIR604 corn plants. Following purification from *E. coli*, dialysis and lyophilization, the resulting sample, designated test substance MCRY3A-0102, was estimated by ELISA to contain ca. 90.3% mCry3A protein by weight. Side-by-side comparisons of mCry3A in test substance MCRY3A-0102 with mCry3A extracted from Event MIR604-derived corn plants indicated that mCry3A from both sources is biologically active against the same target pest species, has the same apparent molecular weight by SDS-PAGE, immunoreacts with the same anti-Cry3A antibody, and is not apparently post-translation. Additionally, peptide mapping of 40% of the mCry3A polypeptide by mass-spectral analysis confirmed the identity and intended amino sequence of mCry3A in test substance MCRY3A-0102. Nucleotide sequencing of the entire DNA insert in Event MIR604-derived plants also confirmed that the mCry3A protein produced in the plants has the intended amino acid sequence. These data justify the use of test substance MCRY3A-0102 in safety studies as a surrogate for mCry3A as produced in Event MIR604-derived plants.

An acute toxicity study was conducted in mice according to EPA's Test Harmonized Guideline OPPTS 870.1100. Test substance MCRY3A-0102 was administered orally by gavage to five male and five female mice at a dose of 2,632 milligrams/kilogram (mg/kg) body weight, representing ca. 2,377 mg of pure mCry3A protein/kg body weight. A negative control group (five males and five females) concurrently received the dosing vehicle alone, an aqueous suspension of 1% methylcellulose, at the same dosing volume used for the test substance mixture. No test substance-related mortalities or clinical signs of toxicity occurred during the 14-day study. One female mouse in the test group was euthanized the day following dosing due to adverse clinical signs resulting from a dosing injury (confirmed by post-mortem examination). At study termination, macroscopic and

microscopic examination of all major organs of the surviving mice revealed no treatment-related abnormalities. Body weight, body weight gain and organ weights (brain, liver, kidneys, and spleen) were comparable in the control and test groups. There was no evidence of toxicity. Accordingly, the LD<sub>50</sub> value for MCRY3A-0102 in male and female mice is greater than 2,632 mg/kg body weight, and the LD<sub>50</sub> value for pure mCry3A protein is greater than 2,377 mg/kg body weight, the single dose tested.

Extensive bioinformatics searches of public protein data bases revealed that the mCry3A protein shows no significant amino acid homology to proteins known to be mammalian toxins or known or suspected to be human allergens. Additional information and testing indicate that the mCry3A protein does not have properties that would suggest it has the potential to become a food allergen. The source of native Cry3A protein (*Bacillus thuringiensis*) is not known to produce food allergens. Unlike allergenic proteins, which typically are present at 1–80% of the total protein in an offending food, the average mCry3A concentration measured in raw grain derived from Event MIR604 corn represents less than 0.0001% of the total protein. This calculation is based on corn grain containing 10% total protein by weight, and assumes less than 1 ppm mCry3A in the grain. Additionally, due to degradation *via* food processing methods, mCry3A will not likely be present in processed food products, or will be present in only trace quantities. The mCry3A protein produced in transformed corn plants is not targeted to a cellular pathway for glycosylation, and shows no evidence of post-translational glycosylation. Bioactivity of mCry3A is lost upon heating at 95 °C for 30 minutes. Upon exposure to simulated mammalian gastric fluid containing pepsin, mCry3A rapidly degrades.

The native Cry3A protein has had a history of safe use as a component of spore preparations of the microbial insecticide *Bacillus thuringiensis* subsp. *tenebrionis*, as an encapsulated component of a microbial insecticide derived from *Bacillus thuringiensis* subsp. *san diego*, and as a plant-incorporated protectant in *Bacillus thuringiensis* potato.

The genetic material occurring in the subject plant-incorporated protectant active ingredient has been adequately characterized. This genetic material (*i.e.*, the nucleic acids DNA and RNA), including regulatory regions, necessary for the production of mCry3A in all corn

will not present a dietary safety concern. “Regulatory regions” are the DNA sequences such as promoters, terminators, and enhancers that control the expression of the genetic material encoding the protein. Based on the ubiquitous occurrence and established safety of nucleic acids in the food supply, a tolerance exemption under the FFDCRA regulations has been established for residues of nucleic acids that are part of plant-incorporated protectants in 40 CFR 174.475 (66 FR 37817 July 19, 2001) (FRL-6057-5). Therefore, no mammalian toxicity is anticipated from dietary exposure to the genetic material necessary for the production of mCry3A protein in all corn.

#### D. Aggregate Exposure

1. *Dietary exposure*—i. *Food*. Average mCry3A levels measured in grain from Event MIR604-derived hybrid field corn plants were less than 1 ppm on a dry-weight or fresh-weight basis. Processed corn products or by-products used in food are unlikely to have measurable mCry3A protein, or will have only trace amounts. Oral exposure is not expected to result in adverse health effects, because of a demonstrated lack of toxicity to mammals and the rapid digestibility of the mCry3A protein. It is expected that any mCry3A protein consumed will be digested as conventional dietary protein.

ii. *Drinking water*. Little to no exposure *via* drinking water is anticipated. Due to the demonstrated mammalian safety profile of mCry3A, such exposure would not present a risk.

2. *Non-dietary exposure*. Non-dietary exposure is not anticipated, due to the proposed use pattern of the product. Exposure *via* dermal or inhalation routes is unlikely because the active ingredient is contained within plant cells. However, if exposure were to occur by non-dietary routes, no risk would be expected because the mCry3A protein is not toxic to mammals.

#### E. Cumulative Exposure

Because there is no indication of mammalian toxicity of the mCry3A protein or the genetic material necessary for its production, it is reasonable to conclude that there will be no cumulative effects for this active ingredient.

#### F. Safety Determination

1. *U.S. population*. The lack of mammalian toxicity at high levels of exposure to the mCry3A protein demonstrates the safety of the product at levels well above possible maximum exposure levels anticipated *via* consumption of all food commodities

produced from corn plants that produce mCry3A. Moreover, little to no human dietary exposure to mCry3A protein is expected to occur via transformed corn. Due to the digestibility and lack of toxicity of the mCry3A protein, and its very low potential to become an allergen in food, dietary exposure is not anticipated to pose any harm for the U.S. population. No special safety provisions are applicable for consumption patterns or for any population sub-groups.

2. *Infants and children*. Based on the mammalian safety profile of the active ingredient and the proposed use pattern, there is ample evidence to conclude with a reasonable certainty that no harm will result to infants and children.

#### G. Effects on the Immune and Endocrine Systems

The active ingredient is derived from sources that are not known to exert an influence on the endocrine or immune systems.

#### H. Existing Tolerances

The registrant is not aware of any existing tolerances or tolerance exemptions for mCry3A protein and the genetic material necessary for its production as an active ingredient.

However, exemptions from tolerances exist for use of the native form of Cry3A protein as a plant-incorporated protectant in Bt potato (40 CFR 180.1147) and as a component of an encapsulated *Bacillus thuringiensis* microbial insecticide (40 CFR 180.1108).

#### I. International Tolerances

No Codex maximum residue levels exists for the plant-incorporated protectant modified Cry3A *Bacillus thuringiensis* protein and the genetic material necessary for its production in corn.

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## ENVIRONMENTAL PROTECTION AGENCY

[FRL-7807-9]

### Receipt of an Application From the State of Ohio to Declare Its Waters of Lake Erie a No Discharge Zone for Vessel Sewage

**AGENCY:** Environmental Protection Agency (EPA).

**ACTION:** Receipt of a petition from the State of Ohio for determination as to the adequacy of facilities on Lake Erie for the disposal of vessel sewage.