Title

Petition to Amend 40 CFR §174.453 to Establish Permanent Exemption from the Requirement of a Tolerance for *Bacillus thuringiensis* Cry1A.105 Insecticidal Protein and the Genetic Material Necessary for Its Production When Used as Plant-incorporated Protectant in All Crops and Agricultural Commodities.

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Date

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Submitted By

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Introduction

On June 29, 2006 EPA granted a temporary exemption from the requirement of a tolerance for the plant-incorporated protectant, *Bacillus thuringiensis* Cry1A.105 protein and the genetic material necessary for its production in food and feed commodities of field corn, sweet corn, and popcorn (40 CFR §174.453). This temporary exemption is valid until June 30, 2009. On November 14, 2006 Monsanto submitted petition to amend 40 CFR §174.453 to remove the time limitation from the exemption from the requirement of a tolerance for the Cry1A.105 protein (Pesticide Petition Number 6F7142). Full characterization and safety assessment of the Cry1A.105 protein has been reviewed by EPA as part of the petition to establish a temporary tolerance exemption and additional reports were submitted as part of the application to register MON 89034 corn in September 2006.

By the way of this petition, Monsanto is requesting EPA to amend 40 CFR §174.453 to establish permanent exemption from the requirement of a tolerance for the Cry1A.105 protein and genetic material necessary for its production in all crops and agricultural commodities. Monsanto consents to publication of this summary as part of the Agency's notice of filing in the Federal Register and as a part of a proposed regulation.

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Section A: The Name, Chemical Identity, and Composition of the Plant-incorporated Protectant

The *Bacillus thuringiensis* protein, Cry1A.105, when used as a plant-incorporated protectant, is the subject of this petition requesting an exemption from the requirement of a tolerance. This exemption would include the genetic material necessary for the production of Cry1A.105 in all crops and agricultural commodities. Reports describing characterization of the Cry1A.105 protein are listed below.

MRID 46694604 Goertz, B., T. Ganguly, J. Lee, and E.A. Rice. 2005. Characterization of the Cry1A.105 Protein Purified from the Corn Grain of MON 89034 and Comparison of the Physicochemical and Functional Properties of the Plant-Produced and *E. coli*-Produced Cry1A.105 Proteins. Monsanto Technical Report MSL-19960.

MRID 46694601 Bogdanova, N.N. 2005. Structural and Functional Similarity of the Cry1A.105 Insecticidal Protein to Cry1A Class of *Bacillus thuringiensis* Proteins. Monsanto Technical Report 05-RA-62-01.

MRID 46951413 MacRae, T.C., C.R. Brown, and S.L. Levine. 2006. Spectrum of Insecticidal Activity of *Bacillus thuringiensis* Cry1A.105 Protein. Monsanto Technical Report MSL-20230.

Section B: The Amount, Frequency, and Time of Application of the Plant-incorporated Protectant

The Cry1A.105 protein is a plant-incorporated protectant, therefore it is not applied to plants. The protein has been found to be present in corn plants throughout their development and in all tested tissues. The following report describing protein levels in MON 89034 tissues was submitted for review by the Agency in September 2006 as part of the application to register MON 89034 corn:

MRID 46951403 Hartmann, A.J., K.E. Niemeyer, and A. Silvanovich. 2006. Assessment of the Cry1A.105 and Cry2Ab2 Protein Levels in Tissues of Insect-Protected Corn MON 89034 Produced in 2005 U.S. Field Trials. Monsanto Technical Report MSL-20285.

Section C: Full Reports of Investigations Made with Respect to the Safety of the Plant-incorporated Protectant

Cry proteins are widely used as topical bioinsecticides and in biotechnology-derived crops. Functional activity of these proteins has been shown to be specific to insects and, therefore, toxicity to mammals is not expected. Detailed human and animal safety assessments and several years of safe human and animal consumption of *Bt* crops confirm their safety. Reports described below were either reviewed by the Agency as part of the petition for temporary tolerance exemption or submitted to the Agency as part of the application to register MON 89034 corn.

MRID 46694603 Bonnette, K.L. 2005. An Acute Oral Toxicity Study in Mice with Cry1A.105 protein. Monsanto study number CRO-2005-050.

MRID 46694606 Kapadia, S.A. and E.A. Rice. 2005. Assessment of the *In vitro* Digestibility of the Cry1A.105 Protein in Simulated Gastric Fluid. Monsanto Technical Report MSL-19929.

MRID 46951408 Kapadia, S. and E.A. Rice. 2005. Assessment of the *in vitro* Digestibility of the Cry1A.105 Protein in Simulated Intestinal Fluid. Monsanto Technical Report MSL-19930.

MRID 46694605 McCoy, R.L. and A. Silvanovich. 2005. Bioinformatics Analysis of the Cry1A.105 Protein Utilizing the AD5, TOXIN5, and ALLPEPTIDES Databases. Monsanto Technical Report MSL-19686.

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

MRID 46694607 Goley, M.E. and J.J. Thorp. 2005. Immunodetection of Cry2Ab2 and Cry1A.105 Proteins in Corn Grain from MON 89034 Following Heat Treatment. Monsanto Technical Report MSL-19899.

Section D: The Results of Tests on the Amount of Plant-incorporated Protectant Remaining, Including a Description of the Analytical Method Used

Cry1A.105 used as plant-incorporated protectant has been found to be present at a range of levels in all tissues of developing corn plants. The results of studies quantifying these levels in corn plants have been submitted to the Agency. A validated ELISA method developed to detect Cry1A.105 protein is described in the report MRID 46951403. In addition, an analytical method for detection of the Cry2Ab2 proteins is available commercially,[1] and a Monsanto-validated analytical method and results of analyses of several corn hybrids containing MON 89034 has also been reviewed by EPA:

MRID 46694503	Dudin,Y. A. and P. Chinnadurai. 2005. Qualitative Detection Method for the Cry2Ab2 Protein in Corn Leaf and Seed of MON 89034 and MON 89597. Monsanto Technical Report 05-RA-39-04.
MRID 46951403	Hartmann, A.J., K.E. Niemeyer, and A. Silvanovich. 2006. Assessment of the Cry1A.105 and Cry2Ab2 Protein Levels in Tissues of Insect-Protected Corn MON 89034 Produced in 2005 U.S. Field Trials. Monsanto Technical Report MSL-20285.

[1] QuickStix™ Combo Kit, Envirologix, Cat. # AS 012 LS

Section E: Practical Methods for Removing Plant-incorporated Protectant that Exceeds any Proposed Tolerance

Such methods are not required because an exemption from the requirement of a tolerance is being sought.

Section F: Proposed Tolerances for the Plant-incorporated Protectant if Tolerances are Proposed

A permanent exemption from the requirement of a tolerance for Cry1A.105 protein and the genetic material necessary for its production in all crops and food and feed agricultural commodities is being sought. Monsanto proposes the following amendment to 40 CFR §174.453 to remove time limitation:

§174.453 *Bacillus thuringiensis* Cry1A.105 protein and the genetic material for its production in corn; exemption from the requirement of a tolerance.

Bacillus thuringiensis Cry1A.105 protein and the genetic material necessary for its production in all crops is exempt from the requirement of a tolerance when used as plant-incorporated protectant in all agricultural commodities. "*Genetic material necessary for its production"* means the genetic material encoding the Cry1A.105 protein and its regulatory regions. "*Regulatory regions"* are the genetic material, such as promoters, terminators, and enhancers that control the expression of the genetic material encoding the Cry1A.105 protein.

Section G: Reasonable Grounds in Support of the Petition

Reasonable grounds to support this petition for an exemption from the requirement of a tolerance for *Bacillus thuringiensis* Cry1A.105 protein and the genetic material necessary for its production in all crops and agricultural commodities when used as plant-incorporated protectant are presented in Section C.

Attachment I. Applicant's Notice of Filing Summary

A summary of this pesticide petition for an exemption from the requirement of a tolerance for residues of *Bacillus thuringiensis* Cry1A.105 protein and the genetic material necessary for its production in all crops and agricultural commodities is combined with the application to register MON 89034 corn submitted in September 2006. Monsanto consents to the use of all or part of this summary by the Agency in preparing the notice of filing and the proposed or final regulation.

An electronic copy of this petition summary in Word format is also being provided on a CD-ROM.

EPA BIOPESTICIDES AND POLLUTION PREVENTION DIVISION
COMPANY NOTICE OF FILING TEMPLATE FOR PESTICIDE PETITIONS
PUBLISHED IN THE FEDERAL REGISTER
(03/09/2007)

EPA Biopesticides and Pollution Prevention Division contact: Mike Mendelsohn, telephone number: 703-308-8715

COMMENT 1] **Monsanto Company**

[COMMENT 2] **[Insert petition number]**

[COMMENT 3] EPA has received a pesticide petition **[insert petition number]** from Monsanto Company, 800 North Lindbergh Blvd., St. Louis, MO 63167, proposing pursuant to section 408(d) of the Federal Food, Drug, and Cosmetic Act (FFDCA), 21 U.S.C. 346a(d), to amend 40 CFR part 180

[COMMENT 4] NA-Remove

[COMMENT 5] NA-Remove

[COMMENT 6] to remove time limitation from the temporary exemption from the requirement of a tolerance for the

[COMMENT 7] NA-Remove

[COMMENT 8] NA-Remove

[COMMENT 9] plant-incorporated protectant *Bacillus thuringiensis* Cry1A.105 protein and the genetic material necessary for its production in all crops and agricultural commodities. EPA is being petitioned to establish a permanent tolerance exemption as part of the registration of insect-protected corn MON 89034.

[COMMENT 10] Pursuant to section 408(d)(2)(A)(i) of the FFDCA, as amended, Monsanto Company has submitted the following summary of information, data, and arguments in support of their pesticide petition. This summary was prepared by Monsanto Company; The summary may have been edited by EPA to clarify terminology, remove extraneous material, or if the summary unintentionally made the reader conclude that the findings reflected EPA's position and not the position of the petitioner.

[COMMENT 11] I. **Monsanto Company Petition Summary**

[COMMENT 12] **[Insert petition number]**

[COMMENT 13] *A. Product name and Proposed Use Practices*

[COMMENT 14] Corn has been genetically modified to produce *Bacillus thuringiensis* (*Bt*) Cry1A.105 and Cry2Ab2 proteins. Plants producing these proteins are derived from transformation events that contain the insecticidal proteins and the genetic material necessary for their expression in corn (vector ZMIR245). The combination of Cry2Ab2 and Cry1A.105 proteins provides a broad spectrum of activity against lepidopteran insect pests and a tool for management of potential insect resistance. The Cry1A.105 protein is closely related to the Cry1A class of proteins, which has well-established human and animal safety. Both the Cry1A.105 and Cry2Ab2 proteins have been fully characterized and no adverse effects were observed for human health and environmental safety.

[COMMENT 15] *B. Product Identity/Chemistry*

[COMMENT 16] 1. **[COMMENT 17]** *Identity of the pesticide and corresponding residues.*

[COMMENT 18] The Cry1A.105 protein is 93.6, 90.0, and 76.7 % identical on the amino acid level to the Cry1Ac, Cry1Ab, and Cry1F proteins, respectively. The gene encoding Cry1A.105 was constructed by combining the nucleotide sequences that encode domains I and II of Cry1Ab, domain III of Cry1F, and substantially all of the C-terminal domain of Cry1Ac. Comparison of the Cry1A.105 sequence to other Cry proteins on the *Bt* phylogenetic tree demonstrated that Cry1A.105 is located in the same cluster as Cry1Ac and is also related to Cry1Ab. The Cry1A.105 protein was fully characterized and equivalence between *in planta*- and *E. coli*-produced proteins used in the safety assessment was demonstrated.

[COMMENT 19] 2. **[COMMENT 20]** *Magnitude of residue at the time of harvest and method used to determine the residue.*

[COMMENT 21] Cry1A.105 residue data are not required for an assessment of human health effects because of the lack of mammalian toxicity.

[COMMENT 22] 3. **[COMMENT 23]** *A statement of why an analytical method for detecting and measuring the levels of the pesticide residue is not needed.*

[COMMENT 24] An analytical method is not required because this petition requests an exemption from tolerances. However, a validated ELISA method for detection of

Cry1A.105 protein and analytical methods for detection of the Cry1Ab and Cry2Ab2 proteins are available and allow for identification of MON 89034 corn.

[COMMENT 25] *C. Mammalian Toxicological Profile*
Cry proteins have been used safely and effectively as pest control agents in microbial *Bt* formulations for more than 45 years. The numerous toxicology studies conducted with these microbial products show no significant adverse effects, and demonstrate that the products are nontoxic to mammals.

Data have been submitted demonstrating the lack of mammalian toxicity at high levels of exposure to purified Cry1A.105 protein. These data demonstrate the safety of the protein at levels well above maximum possible exposure levels that are reasonably anticipated in crops. This conclusion is consistent with the Agency position regarding toxicity and residue data requirements for the microbial *Bt* products which utilize the same proteins as these plant-incorporated protectants (40 CFR §158.740(b)(2)(i)). For microbial products, further toxicity and residue testing are only triggered by significant acute effects in studies such as the mouse oral toxicity study.

When proteins are toxic, they are known to act via acute mechanisms and at very low levels (Sjoblad et al. "Toxicological Considerations for Protein Components of Biological Pesticide Products." *Reg. Toxicol. Pharmacol.* 15:3-9, 1992). Acute oral toxicity study has been evaluated for the Cry1A.105 protein. Furthermore, amino acid sequence comparisons of the Cry1A.105 protein to known toxic proteins showed no similarities.

Because Cry1A.105 is a protein, the potential for allergic sensitivities was evaluated. Common food allergens are present at high concentrations in food, are resistant to pepsin digestion, may be resistant to acid or heat, and can be glycosylated. Data have been submitted demonstrating that the Cry1A.105 protein is rapidly degraded by simulated gastric fluid *in vitro*. In a solution of simulated gastric fluid, complete degradation of the detectable Cry1A.105 protein occurred within 30 seconds of incubation.

An analysis of amino acid sequences of known allergens uncovered no evidence of sequence homology with the Cry1A.105 protein, even at the level of eight contiguous amino acid residues.

The genetic material encoding the Cry1A.105 protein and the regulatory regions controlling expression of the *cry1A.105* gene are nucleic acids (DNA and RNA). DNA and RNA occur in all forms of plant and animal life and there is no documented instance of nucleic acids being associated with toxic effects when consumed as a component of food. DNA and RNA are in the category of substances generally considered as safe (GRAS). EPA exempts nucleic acids from the FFDCA Section 408 requirements. Data

characterizing the genetic material necessary for the production of Cry1A.105 in corn were provided to the Agency. No mammalian toxicity is anticipated from dietary exposure to the genetic material necessary for production of the subject plant-incorporated protectant.

[COMMENT 26] *D. Aggregate Exposure*

[COMMENT 27] 1. **[COMMENT 28]** *Dietary exposure*

[COMMENT 29] Cry1A.105 is a plant-incorporated protectant in corn, thus dietary exposure is deemed to be the most relevant route for assessing human risk.

[COMMENT 30] i. **[COMMENT 31]** *Food*

[COMMENT 32] Monsanto has considered available information on the aggregate exposure levels to consumers and major identifiable groups of consumers to the protein residue and related substances. These considerations include dietary exposure under the tolerance exemptions in effect for the plant-incorporated protectant residue, and exposure from non-occupational sources. Oral exposure may occur at very low levels from ingestion of processed corn products. However, a lack of mammalian toxicity and the rapid digestibility of the plant-incorporated protectants have been demonstrated.

[COMMENT 33] ii.**[COMMENT 34]** *Drinking water*

[COMMENT 35] Oral exposure from ingestion of drinking water is unlikely because the proteins are present at low levels within the plant and previously submitted studies demonstrate that Cry1A.105 protein is rapidly degraded in soil and, therefore, is not present in drinking water.

[COMMENT 36] 2. **[COMMENT 37]** *Non-dietary exposure*

[COMMENT 38] Exposure via skin or inhalation is not likely because the plant-incorporated protectants are contained within plant cells, which essentially eliminates these exposure routes or reduces them to negligible levels. The use sites for the Cry1A.105 protein are all agricultural for control of insects. Exposure to workers and bystanders resulting from plant pesticides will be negligible and unlikely to add measurably to any exposure resulting from microbial or other *Bacillus thuringiensis* formulations. Exposure to infants and children via residential or lawn use is not expected.

[COMMENT 39] *E. Cumulative Exposure*

[COMMENT 40] Because there is no indication of mammalian toxicity to the plant-incorporated protectants there will be no opportunity for cumulative toxic effects.

[COMMENT 41] *F. Safety Determination*

[COMMENT 42] 1. **[COMMENT 43]** *U.S. population*

[COMMENT 44] Sufficient data have been submitted to assess the health risk of Cry1A.105 protein and the genetic material necessary for its production in all raw agricultural commodities. The absence of toxicity in high dose acute oral studies, the lack of sequence homology with known protein toxins, rapid digestion in a gastric matrix, and minimal allergenicity potential provide a reasonable certainty of no harm for the U.S. general population potentially exposed to the Cry1A.105 protein.

[COMMENT 45] 2. **[COMMENT 46]** *Infants and children*

[COMMENT 47] Non-dietary exposure to infants and children is not anticipated due to the patterns of use for these plant-incorporated protectants. The submitted data provide no evidence of adverse threshold effects for the Cry1A.105 protein that would warrant application of an additional safety factor for the protection of infants and children. Furthermore, the provisions for consumption patterns, special susceptibility, and cumulative effects do not apply.

[COMMENT 48] *G. Effects on the Immune and Endocrine Systems*

[COMMENT 49] The lack of Cry1A.105 homology to known toxins and allergens and their rapid degradation in a mammalian digestive system suggests minimal risk for adverse effects on the immune system. These pesticidal active ingredients are proteins derived from sources that are not known to exert an influence on the endocrine system.

[COMMENT 50] *H. Existing Tolerances*

[COMMENT 51] The *Bacillus thuringiensis* Cry1A.105 protein and the genetic material necessary for its production in corn is exempt from the requirement of a tolerance when used as a plant incorporated protectant in food and feed commodities of field corn, sweet corn, and popcorn until June 30, 2009 (40 CFR§174.453).

[COMMENT 52] *I. International Tolerances*

[COMMENT 53] No Codex maximum residue levels have been established for these plant-incorporated protectants at this time.