

its production in potatoes. The Monsanto Co. requested this exemption from the requirement of a tolerance. This regulation eliminates the need to establish a maximum permissible level for residues of this plant pesticide in potatoes.

**EFFECTIVE DATE:** Effective on May 3, 1995.

**ADDRESSES:** Written objections and hearing requests, identified by the document control number, [PP 3F4273/R2132], may be submitted to: Hearing Clerk (1900), Environmental Protection Agency, Rm. M3708, 401 M St., SW., Washington, DC 20460. A copy of any objections and hearing requests filed with the Hearing Clerk should be identified by the document control number and submitted to: Public Response and Program Branch, Field Operations Division (7506C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. In person, bring copy of objections and hearing requests to: Rm. 1132, CM #2, 1921 Jefferson Davis Hwy., Arlington, VA 22202. Fees accompanying objections shall be labeled "Tolerance Petition Fees" and forwarded to: EPA Headquarters Accounting Operations Branch, OPP (Tolerance Fees) P.O. Box 360277M, Pittsburgh, PA 15251.

A copy of objections and requests for hearings filed with the Hearing Clerk may also be submitted electronically by sending electronic mail (e-mail) to: opp-docket@epamail.epa.gov. Copies of objections and requests for hearings must be submitted as an ASCII file avoiding the use of special characters and any form of encryption. Copies of objections and requests for hearings will also be accepted on disks in WordPerfect in 5.1 file format or ASCII file format. All copies of objections and requests for hearings in electronic form must be identified by the docket number [PP 3F4273/R2132]. No Confidential Business Information (CBI) should be submitted through e-mail. Electronic copies of objections and requests for hearings on this rule may be filed online at many Federal Depository Libraries. Additional information on electronic submissions can be found below in this document.

**FOR FURTHER INFORMATION CONTACT:** By mail: Willie H. Nelson, Biopesticides and Pollution Prevention Division, Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. Office location and telephone number: Rm. 51B6, CS #1, 2800 Crystal Drive, Arlington, VA 22202, (703)-308-8128; e-mail: nelson.willie@epamail.epa.gov.

**SUPPLEMENTARY INFORMATION:** EPA issued a notice, published in the **Federal Register** of December 8, 1993 (58 FR 64583), which announced that the Monsanto Co., 700 Chesterfield Village Parkway, St. Louis, MO 63198, had submitted a pesticide petition, PP 3F4273, to EPA requesting that the Administrator, pursuant to section 408(d) of the Federal Food, Drug and Cosmetic Act (FFDCA), 21 U.S.C. 346a(d), establish an exemption from the requirement of a tolerance for the plant pesticide *Bacillus thuringiensis* subsp. *tenebrionis* (B.t.t) Colorado potato beetle (CPB) control protein (CryIIIa).

EPA has assigned the active ingredient of this product the name *Bacillus thuringiensis* CryIIIa delta-endotoxin and the genetic material necessary for its production. "Genetic material necessary for production" means the CryIIIa gene and its regulatory regions. "Regulatory regions" are the genetic material that control the expression of the gene, such as promoters, terminators, and enhancers.

Monsanto has genetically modified potato plants to produce the pesticidal protein derived from the common soil bacterium *Bacillus thuringiensis* subsp. *tenebrionis*. The protein produced by CPB-resistant potatoes is identical to that found in nature. Monsanto has genetically engineered potatoes by using plant-expressed vectors that transferred the CryIIIa and neomycin phosphotransferase II (nptII) marker gene into the genomic DNA of the potato plants. In the **Federal Register** of September 28, 1994 (59 FR 49353), EPA exempted nptII and the genetic material necessary for its production in or on all raw agricultural commodities when used as an inert. There were no adverse comments or requests for referral to an advisory committee received in response to the notice of filing of the petition, PP 3F4273 (58 FR 64582, Dec. 8, 1993).

#### Residue Chemistry Data

Residue chemistry data were not required because of the lack of toxicity to this active ingredient. This is similar to the Agency position regarding the submission of residue data for the microbial *Bacillus thuringiensis* products from which this plant pesticide was derived. (See 40 CFR 158.740(b).) For microbial products, residue data are required only when Tier II or III toxicology data are required. The kinds of studies submitted for this plant pesticide are like those in Tier I, not Tiers II or III. Submitted data indicated that the product is of low mammalian toxicity/pathogenicity and

the kinds of studies required in Tier II or III were not appropriate. Therefore, no residue data are required in order to grant an exemption from the requirement of a tolerance for Monsanto's plant pesticide, *Bacillus thuringiensis* Cry IIIa delta-endotoxin protein, the CryIIIa gene and the genetic material necessary for its production in potato.

#### Product Analysis

Monsanto submitted information which adequately described the CryIIIa delta-endotoxin from B.t.t., as expressed in potato, along with the genetic material necessary for its production. Because it would be difficult, or impossible, to extract sufficient biologically active toxin from the plants to perform toxicology tests, Monsanto used delta-endotoxin produced in bacteria. Product analysis data were submitted to show that the microbially expressed and purified CryIIIa delta-endotoxin is sufficiently similar to that expressed in the plant to be used for mammalian toxicological purposes.

1. *Molecular characterization of CPB-resistant Russet Burbank Potatoes equivalence of microbially produced B.t.t. protein.* The relative size and number of copies of the DNA inserted into potatoes was demonstrated with endonuclease digested chromosomal DNA from field-grown potato plants southern blotted with the entire introduced plasmid PV-STBT02 as the probe. These southern blots provided information about the number of copies of introduced DNA, the lack of significant amount of DNA introduced outside the border regions, and integrity of the introduced DNA near the endonuclease cut site. These results indicate only that the DNA necessary to produce the CryIIIa delta endotoxin were introduced into the plant, thus indicating that exposure would only be to the CryIIIa delta-endotoxin and the nucleic acids found in the genetic material necessary for its production. Such nucleic acids have not, by themselves, been associated with toxic effects to animals or humans and are regular constituents of the human diet.

2. *Equivalence of microbially produced and plant-produced B.t.t. protein also called Colorado potato beetle active protein from Bacillus thuringiensis subsp. tenebrionis.* Microbially produced delta endotoxin from the CryIIIa gene as expressed in *Escherichia coli* and in potato tubers were compared. The data consists of SDS-PAGE comigration, Western blot analysis, staining for carbohydrate residues, N-terminal amino acid sequence analysis, and biological