

several reasons: the duration of the event is limited; the event is at a late hour; the zone is located within a Federal Anchorage and does not impact a navigable channel; vessel traffic may safely pass to the east of this area; and the extensive, advance advisories which will be made. Accordingly, the Coast Guard expects the economic impact of this regulation to be so minimal that a Regulatory Evaluation is unnecessary.

Small Entities

Under the Regulatory Flexibility Act (5 U.S.C. 601 et seq.), the Coast Guard must consider whether this regulation will have a significant economic impact on a substantial number of small entities. "Small entities" include independently owned and operated small businesses that are not dominant in their field and that otherwise qualify as "small business concerns" under Section 3 of the Small Business Act (15 U.S.C. 632).

For the reasons set forth in the Regulatory Evaluation, the Coast Guard expects the impact of this regulation to be minimal. The Coast Guard certifies under 5 U.S.C. 605(b) that this regulation will not have a significant economic impact on a substantial number of small entities.

Collection of Information

This regulation contains no collection of information requirements under the Paperwork Reduction Act (44 U.S.C. 3501).

Federalism

The Coast Guard has analyzed this action in accordance with the principles and criteria contained in Executive Order 12612 and has determined that this regulation does not raise sufficient federalism implications to warrant the preparation of a Federalism Assessment.

Environment

The Coast Guard has considered the environmental impact of this regulation and concluded that under section 2.B.2.e. of Commandant Instruction M16475.1B, revised 59 FR 38654, July 29, 1994, the promulgation of this regulation is categorically excluded from further environmental documentation. A Categorical Exclusion Determination and Environmental Analysis Checklist are included in the docket. Under the National Environmental Policy Act, the approval of the permit for marine event for this event is a federal action which is categorically excluded in accordance with section 2.B.2.e(35)(h) of Commandant Instruction M16475.1B. This fireworks display lasts less than 30

minutes and is expected to involve less than 200 spectator craft.

List of Subjects in 33 CFR Part 165

Harbors, Marine safety, Navigation (water), Reporting and recordkeeping requirements, Security measures, Waterways.

For reasons set out in the preamble, the Coast Guard amends 33 CFR Part 165 as follows:

PART 165—[AMENDED]

1. The authority citation for Part 165 continues to read as follows:

Authority: 33 U.S.C. 1231; 50 U.S.C. 191; 33 CFR 1.05-1(g), 6.04-1, 6.04-6, and 160.5; 49 CFR 1.46.

2. A temporary section 165.T01-138, is added to read as follows:

§ 165.T01-138 Safety Zone; Periphonics Corporation 25th Anniversary Fireworks, Upper New York Bay, New York.

(a) *Location.* The safety zone includes all waters of Upper New York Bay, within a 300 yard radius of the fireworks barge anchored approximately 300 yards east of Liberty Island, New York, at approximately 40°41'18" N latitude, 074°02'25" W longitude (NAD 1983).

(b) *Effective period.* This section is in effect from 10 p.m. until 11:20 p.m. on September 16, 1995, unless extended or terminated sooner by the Captain of the Port New York.

(c) *Regulations.* (1) The general regulations contained in 33 CFR 165.23 apply.

(2) All persons and vessels shall comply with the instructions of the Coast Guard Captain of the Port or the designated on scene patrol personnel. U.S. Coast Guard patrol personnel include commissioned, warrant, and petty officers of the Coast Guard. Upon being hailed by a U.S. Coast Guard vessel via siren, radio, flashing light, or other means, the operator of a vessel shall proceed as directed.

Dated: September 5, 1995.

T.H. Gilmour,

Captain, U.S. Coast Guard, Captain of the Port New York.

[FR Doc. 95-22983 Filed 9-14-95; 8:45 am]

BILLING CODE 4910-14-M

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 180

[PP 4F4331/R2170; FRL-4976-9]

RIN 2070-AB78

Plant Pesticide *Bacillus Thuringiensis* CryIA(c) Delta-Endotoxin and the Genetic Material Necessary for Its Production in Cotton; Tolerance Exemption

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This rule establishes an exemption from the requirement of a tolerance for residues of the plant pesticide active ingredient *Bacillus thuringiensis* CryIA(c) delta-endotoxin and the genetic material necessary for its production in cotton. The Monsanto Co. requested the exemption from the requirement of a tolerance under the Federal Food, Drug and Cosmetic Act. The rule eliminates the need to establish a maximum permissible level for residues of this plant pesticide in cotton.

EFFECTIVE DATE: September 15, 1995.

ADDRESSES: Written objections and hearing requests, identified by the document control number [PP 4F4331/R2170] 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 hearing requests 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 hearing requests must be submitted as an ASCII file avoiding the use of special characters and any form of encryption. Copies of objections and hearing requests will also be accepted

on disks in WordPerfect in 5.1 file format or ASCII file format. All copies of objections and hearing requests in electronic form must be identified by the docket number [PP 4F4331/R2170]. No Confidential Business Information (CBI) should be submitted through e-mail. Electronic copies of objections and hearing requests 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, 2800 Crystal Drive, Arlington, VA 22202, telephone no.: 703-308-8128; e-mail: nelson.willie@epamail.epa.gov.

SUPPLEMENTARY INFORMATION: EPA issued a notice, published in the **Federal Register** of September 14, 1994 (59 FR 47137), which announced that Monsanto Co., 700 Chesterfield Village Parkway, St. Louis, MO 63198, had submitted pesticide petition (PP) 4F4331 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), exempt from the requirement of a tolerance the plant pesticide *Bacillus thuringiensis* var. *kurstaki* delta-endotoxin protein as produced by the CryIA(c) gene and its controlling sequences. EPA has assigned the active ingredient of this product the name *Bacillus thuringiensis* CryIA(c) delta-endotoxin and the genetic material necessary for its production. "Genetic material necessary for production" means the CryIA(c) gene and its regulatory regions. "Regulatory regions" are the genetic materials that control the expression of the gene, such as promoters, terminators, and enhancers. Monsanto has genetically modified cotton plants to produce the pesticidal protein derived from the common soil bacterium *Bacillus thuringiensis*. The protein produced by these cotton plants is identical to that found in nature.

There were no adverse comments or requests for referral to an advisory committee received in response to the notice of filing.

Residue Chemistry Data

Residue chemistry data were not required because of the lack of toxicity to this active ingredient. This position is similar to that the Agency has taken 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 Tier 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 to grant an exemption from the requirements of a tolerance for Monsanto's plant pesticide, *Bacillus thuringiensis* CryIA(c) delta-endotoxin protein, the CryIA(c) gene and the genetic material necessary for its production in cotton.

Product Analysis

Monsanto submitted information which adequately described the CryIA(c) delta-endotoxin from B.t., as expressed in cotton, 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 CryIA(c) delta-endotoxin is sufficiently similar to that expressed in the plant to be used for mammalian toxicological purposes. Plant and microbially produced CryIA(c) delta-endotoxin were shown by these studies to have similar molecular weights and immunoreactivity (SDS-PAGE and Western blots), to lack detectable post-translational modification (glycosylation tests), to have identical amino acid sequences in the N-terminal region and to have similar results in bioassays against *Heliothis virescens* and *Helicoverpa zea*. While it is difficult to prove that two proteins are identical, the combined results of the above studies indicate a high probability that these two sources produce proteins that are essentially identical by available protein analytical assays.

Toxicology Assessment

Toxicity

The delta-endotoxin proteins of *B. thuringiensis* have been intensively studied and no indications of mammalian toxicity have been reported. Furthermore, approximately 176 different *B. thuringiensis* products have been registered since 1961, and the Agency has not received any reports of dietary toxicity attributable to their use.

This is especially significant because FIFRA section 6(a)(2) requires registrants to report any adverse effects to EPA. Therefore, the Agency does not anticipate any mammalian toxicity from this protein in plants based on the use history of *B. thuringiensis* products. The *in vitro* digestibility assay provides useful information to predict the metabolic fate of the CryIA(c) protein and its potential as a food allergen. However, it is not clear how this assay's results relate to protein toxicity. Therefore, the Agency requested that an acute oral toxicity study be done to confirm the expected lack of toxicity indicated by the *in vitro* digestibility results.

Monsanto's submitted oral toxicity data support the prediction that this protein would be nontoxic to humans. CryIA(c) delta-endotoxin was chosen in order to obtain sufficient material for mammalian testing if any exposure were anticipated in food or feed. The *in vitro* digestibility studies indicate that the protein would rapidly be degraded following ingestion.

The genetic material necessary for the production of the *Bacillus thuringiensis* CryIA(c) delta endotoxin are the nucleic acids (DNA and RNA) that constitute the CryIA(c) gene and its controlling sequences. DNA and RNA are common to all forms of life, including plants, and the Agency knows of no instance where these nucleic acids have been associated with toxic effects related to the consumption of food. These ubiquitous nucleic acids as they appear in the subject active ingredient have been adequately characterized by the applicant. Therefore, no mammalian toxicity is anticipated from dietary exposure to the genetic material necessary for the production of the *Bacillus thuringiensis* CryIA(c) delta-endotoxin in cotton.

Allergenicity

Despite decades of widespread use of *Bacillus thuringiensis* as a pesticide (it has been registered since 1961), there have been no confirmed reports of immediate or delayed allergic reactions from exposure. Such incidents, should they occur, are required to be reported under FIFRA section 6(a)(2) and as a data requirement for registration of microbial pesticides (40 CFR 158.740 and Subdivision M of the FIFRA testing guidelines, NTIS # PB89-211676).

Studies done in laboratory animals as reported in the literature also have not indicated any potential for allergic reactions to *B. thuringiensis* or its components, including the delta-endotoxin in the crystal protein. Recent *in vitro* studies also confirm that the

delta-endotoxin would be readily digestible *in vivo*.

Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases, are glycosylated, and are present at high concentrations in the food (Conference on Scientific Issues, Related to Potential Allergenicity in Transgenic Food Crops, April 18 and 19, Annapolis, MD, sponsored by FDA, EPA, and USDA). The delta-endotoxins are not present at high concentrations, are not resistant to degradation by heat, acid and proteases, and are apparently not glycosylated when produced in plants. The company has submitted data to indicate that the CryIA(c) delta-endotoxin is rapidly degraded by gastric fluid *in vitro*, that it is not present as a major component of food, and that it is apparently nonglycosylated when produced in plants.

Submitted Data

1. *Product characterization (431452-01)*. Southern blot analysis restriction digests of DNA extracts from cotton line 531 and the parental Coker 312 showed that there is probably only one insert of the cryIA(c) gene cassette present in the transformed line. The introduced gene appears to be genetically stable in the cotton according to the results of progeny selfing and backcrosses with elite lines. The amino acid sequence is homologous to the cryIA(b) gene from HD-1 for positions 1-466 and homologous to cryIA(c) for positions 467-1178 with a single exception of a leucine-serine 766 in the crystal portion of the protein cleaved prior to toxin activation. Western blot analysis of purified toxin, leaf tissue from cotton line 531 and the parental Coker 312 shows that trypsinized extracts have comigrating bands similar to that found in B.t.k HD-73 protein reference material and commercial preparations. *Classification: Acceptable.*

2. *Product characterization (431452-02)*. B.t.k. HD-73 toxin isolated from either cotton line 531 or 931 were compared to the same toxin expressed in *E. coli* by SDS-PAGE, western blot, glycosylation and bioactivity (Conference on Scientific Issues Related to Potential Allergenicity in Transgenic Food Crops, April 18 and 19, 1994, Annapolis, MD, sponsored by FDA, EPA, and USDA). The data presented suggest the bacterially produced protein and that found in cotton are equivalent and suggest the bacterially produced B.t.k. HD-73 toxin can serve as a surrogate test substance for the toxicological tests to support the registration of transgenic cotton. This initial submission was classified as

supplementary because of the absence of sufficient description of how the B.t.k. HD-73 protein was isolated and purified from the cotton plant. A cursory description is found in "Assessment of Equivalence Between *E. coli*-Produced and Cotton-Produced Btk HD73 Protein * * *." (MRID 431452-02, p.13). Monsanto has since provided complete details regarding isolation and purification. With the clarification of the extraction procedure described above, the product characterization study (MRID 431452-02) has been upgraded from supplementary to acceptable.

Classification: Acceptable.

3. *Product characterization (431452-03)*. The delta-endotoxin from B.t.k. HD-73 (lot 5025385) produced in *E. coli* containing the plasmid pMON10569 was purified, lyophilized and found to have the following characteristics: 4.5% moisture, 75.6% protein (amino acid analysis), 70% protein (BCA), 88% HD-73 specific protein (ELISA), 80% HD-73 specific protein (Coomassie blue PAGE), 1.6 ug gram negative endotoxin/mg and no significant trace metals except for sodium, potassium, and phosphate. The molecular weight of the B.t.k. HD-73 toxin was estimated to be 134.8 kD for the full length species and 77.1 kD for the tryptic. The functional activity was found to be an LC₅₀ of 0.28 ppm against *Heliothis virescens*.

Classification: Acceptable.

4. *Product characterization (431452-04)*. Ten insect pest species from 5 families were tested for their sensitivity to B.t.k. HD-73 protein. Only in the lepidopteran species was there significant mortality. The green peach aphid showed marginal effects from treatment with a tryptic digest of the CryIA(c) toxin from B.t.k. which was not reproducible in a repeat test. The tryptic digest preparation positive control also showed higher mortality in the TBW test.

Classification: Acceptable.

5. *Acute oral toxicity (431452-13)*. Ten male and female CD-1 mice per dose level were exposed by oral gavage to 500, 1,000 and 4,200 mg/kg body weight of *E. coli* produced B.t.k. HD-73 toxin. Controls were given the protein equivalent of 6,340 mg/kg of BSA. No mortalities or treatment related adverse effects were seen in either the treated or control mice. There were no observable dose-related effects seen upon necropsy. *Classification: Acceptable. Tox category IV.*

6. *In vitro digestibility (431452-14)*. The B.t.k. HD-73 protein was rapidly degraded to fragments not recognized in a western blot after 7 minutes incubation in simulated gastric fluid

(SGF) and was not active in a TBW bioassay after SGF incubation. The *in vitro* digestibility assay provides useful information to predict the metabolic fate of the CryIA(c) protein and its potential as a food allergen.

Classification: Acceptable.

Conclusions

In summary, based upon the submitted studies and other available information, the Agency does not foresee any human health hazards from the use of the *Bacillus thuringiensis* CryIA(c) delta-endotoxin and the genetic material necessary for its production.

Based upon submitted data and a review of its use, EPA has found that when used in accordance with good agricultural practice, this ingredient is useful for the purpose for which the tolerance exemption is sought. Based on the information considered, EPA concludes that a tolerance is not necessary to protect the public health. Therefore, the exemption from the requirement of a tolerance is established as set forth below.

Acceptable daily intake (ADI) and maximum permissible intake (MPI) considerations are not relevant to this petition because the data/information submitted demonstrate that this active ingredient is not toxic to mammalian species. No enforcement actions are expected, based upon the toxicity for this plant pesticide. Therefore, the requirement for an analytical method for enforcement purposes is not applicable to this exemption request.

Any person adversely affected by this regulation may, within 30 days after publication of this document in the **Federal Register**, file written objections and/or a request for a hearing with the Hearing Clerk, at the address given above (40 CFR 178.20). A copy of the objections and hearing requests filed with the Hearing Clerk should be submitted to the OPP docket for this rulemaking. The objections submitted must specify the provisions of the regulation deemed objectionable and the grounds for the objections, and must conform to the other requirements of 40 CFR 178.25. Each objection must be accompanied by the fee prescribed by 40 CFR 180.33(i). If a hearing is requested, the objections must include a statement of the factual issue(s) on which a hearing is requested, the requestor's contentions on each such issue, and a summary of any evidence relied upon by the objector (40 CFR 178.27). A request for a hearing will be granted if the Administrator determines that the material submitted shows the following: There is a genuine and substantial issue

of fact; there is a reasonable possibility that available evidence identified by the requestor would, if established, resolve one or more of such issues in favor of the requestor, taking into account uncontested claims or facts to the contrary; and resolution of the factual issue(s) in the manner sought by the requestor would be adequate to justify the action requested (40 CFR 178.32).

A record has been established for this rulemaking under docket number [PP 4F4331/R2170] (including objections and hearing requests submitted electronically as described below). A public version of this record, including printed, paper versions of electronic comments, which does not include any information claimed as CBI, is available for inspection from 8 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The public record is located in Room 1132 of the Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs, Environmental Protection Agency, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA.

Written objections and hearing requests, identified by the document control number [PP 4F4331/R2170], may be submitted to the Hearing Clerk (1900), Environmental Protection Agency, Rm. 3708, 401 M St., SW., Washington, DC 20460.

A copy of electronic objections and hearing requests filed with the Hearing Clerk can be sent directly to EPA at: opp-Docket@epamail.epa.gov

A copy of electronic objections and hearing requests filed with the Hearing Clerk must be submitted as an ASCII file avoiding the use of special characters and any form of encryption.

The official record for this rulemaking, as well as the public version, as described above will be kept in paper form. Accordingly, EPA will transfer any objections and hearing requests received electronically into printed, paper form as they are received and will place the paper copies in the official rulemaking record which will also include all objections and hearing requests submitted directly in writing. The official rulemaking record is the paper record maintained at the address in ADDRESSES at the beginning of this document.

Under Executive Order 12866 (58 FR 51735, Oct. 4, 1993), the Agency must determine whether the regulatory action is "significant" and therefore subject to review by the Office of Management and Budget (OMB) and the requirements of the Executive Order. Under section 3(f), the order defines a "significant regulatory action" as an action that is

likely to result in a rule (1) having an annual effect on the economy of \$100 million or more, or adversely and materially affecting a sector of the economy, productivity, competition, jobs, the environment, public health or safety, or State, local or tribal governments or communities (also referred to as "economically significant"); (2) creating serious inconsistency or otherwise materially altering the budgetary impacts of entitlement, grants, user fees, or loan programs or the rights and obligations or recipients thereof; or (3) raising novel legal or policy issues arising out of legal mandates, the President's priorities, or the principles set forth in this Executive Order.

Pursuant to the terms of the Executive Order, EPA has determined that this rule is not "significant" and is therefore not subject to OMB review.

Pursuant to the requirements of the Regulatory Flexibility Act (Pub. L. 96-354, 94 Stat. 1164, 5 U.S.C. 601-612), the Administrator has determined that regulations establishing new tolerances or raising tolerance levels or establishing exemption from tolerance requirements do not have a significant economic effect on a substantial number of small entities. A certification statement to this effect was published in the **Federal Register** of May 4, 1981 (49 FR 24950).

List of Subjects in 40 CFR Part 180

Environmental protection, Administrative practice and procedure, Agricultural commodities, Pesticides and pests, Reporting and recordkeeping requirements.

Dated: August 31, 1995.

Daniel M. Barolo,

Director, Office of Pesticide Programs.

Therefore, 40 CFR part 180 is amended as follows:

PART 180—[AMENDED]

1. The authority citation for part 180 continues to read as follows:

Authority: 21 U.S.C. 346a and 371.

2. In subpart D, by adding new § 180.1155, to read as follows:

§ 180.1155 *Bacillus thuringiensis* CryIA(c) delta-endotoxin and the genetic material necessary for its production; exemption from the requirement of a tolerance.

Bacillus thuringiensis CryIA(c) delta endotoxin and the genetic material necessary for its production are exempted from the requirement of a tolerance when used as a plant pesticide in cotton. "Genetic material necessary for its production" means the CryIA(c) gene and its regulatory regions.

"Regulatory regions" are the genetic materials that control the expression of the gene, such as promoters, terminators, and enhancers.

[FR Doc. 95-23077 Filed 9-13-95; 12:19 pm]

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DEPARTMENT OF THE INTERIOR

Bureau of Land Management

43 CFR Public Land Order 7159

[AZ-930-1430-01; A-1880, A-12962, A-13003]

Revocation of Coal Land Withdrawals; Arizona

AGENCY: Bureau of Land Management, Interior.

ACTION: Public land order.

SUMMARY: This order revokes in their entirety two Secretarial Orders and two Executive Orders insofar as they affect the remaining 134,960 acres of lands withdrawn for Federal coal classification purposes. The lands are located within the Coronado and Sitgreaves National Forests and the San Carlos Indian Reservation. The withdrawals are no longer needed as the United States Geological Survey has classified the lands as Non-Coal lands and has recommended revocation of the withdrawals. The lands located within the National Forests will be opened to nonmetalliferous mining and to such forms of disposition as may be law be made of National Forest System lands. The lands located within the Indian Reservation will not be opened since reservation lands are not subject to entry under the general land laws or the United States mining laws.

EFFECTIVE DATE: October 16, 1995.

FOR FURTHER INFORMATION CONTACT: John Mezes, BLM Arizona State Office, P.O. Box 16563, Phoenix, Arizona 85011, (602) 650-0518.

By virtue of the authority vested in the Secretary of the Interior by Section 204 of the Federal Land Policy and Management Act of 1976, 43 U.S.C. 1714 (1988), it is ordered as follows:

1. The Secretarial Orders dated November 29, 1909, and December 28, 1909, and the Executive Order dated July 7, 1910, which withdrew lands and created Coal Land Withdrawal, Arizona No. 1, are hereby revoked in their entirety insofar as they affect the remaining withdrawn lands described as follows: