RESPONSE OF THE ENVIRONMENTAL PROTECTION AGENCY TO PETITION FOR RULEMAKING AND COLLATERAL RELIEF CONCERNING THE REGISTRATION AND USE OF GENETICALLY ENGINEERED PLANTS EXPRESSING *BACILLUS THURINGIENSIS* ENDOTOXINS, SUBMITTED BY PETITIONERS GREENPEACE INTERNATIONAL, INTERNATIONAL FEDERATION OF ORGANIC AGRICULTURE MOVEMENTS, INTERNATIONAL CENTER FOR TECHNOLOGY ASSESSMENT, *et al*.

April 19, 2000

INTRODUCTION

The Environmental Protection Agency ("EPA" or "the Agency") hereby responds to the *Petition for Rulemaking and Collateral Relief Concerning the Registration and Use of Genetically Engineered Plants Expressing Bacillus Thuringiensis Endotoxins* ("Petition") filed by Petitioners Greenpeace International, International Federation of Organic Agriculture Movements, International Center for Technology Assessment, Cissy Bowman, Kate Burroughs, Valecia Wadsworth-Carr, California Certified Organic Farmers, Center for Ethics and Toxics, the Edmonds Institute, Farm Verified Organic, Inc., Florida Certified Organic Growers and Consumers, Jim and Mary Gerritsen, Hoosier Organic Marketing Education, Indiana Certified Organic, Inc., Institute for Agricultural and Trade Policy, Integrated Fertility Management, Maine Organic Farmers and Gardeners Association, New York Coalition for Alternatives to Pesticides, Northeast Organic Farming Association, Organic Farmers Information and Education Foundation, Organic Farmers Marketing Association, Texas Organic Growers Association, Rodger and Sandy Sanders, P Marc Schwartz, and Mark Wilks [hereinafter "Petitioners"] on September 16, 1997.[1] In the Petition, Petitioners request the Administrator to undertake the following actions:

> (1) *Declare that the registration of genetically engineered plants that express the pesticide Bacillus thuringiensis (B.t.) cause an unreasonable adverse effect on the environment;*
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> (2) *Cancel the registrations of all genetically engineered plants that express the pesticide B.t. registered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA);*
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> (3) *Cease and desist from undertaking any new registration procedures and/or determinations of registration for any genetically engineered plants that express the pesticide B.t. in any manner;*

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[1] The following petitioners were subsequently added at Petitioners' requests: Arizona Toxics Information; Council for Responsible Genetics; National Campaign Against the Misuse of Pesticides; National Family Farm Coalition; Oregon Tilth Organic Certified; Organic Crop Improvement Association (Arkansas Chapter); Organic Trade Association; Rural Advancement Fund International-USA; Sierra Club; Sustainable Cotton Project; Virginia Association of Biological Farmers; Vreseis Ltd.

The Petition, Petitioners' various addenda to the Petition adding petitioners, public comments on the Petition, all document that may not be generally available that are used in support of EPA's Response to the Petition, and other relevant documents have been placed in the Office of Pesticide Programs Special Docket entitled "Greenpeace Petition Concerning Transgenic *B.t.* Plants."

(4) *Pursuant to 40 C.F.R. Part 154, immediately undertake Special Review procedures for all registered genetically engineered plants that express the pesticide B.t.;*

(5) *Pursuant to 42 U.S.C. § 4332(c), complete a programmatic environmental impact statement (Programmatic EIS) analyzing the agency's major federal action of registering genetically engineered plant-pesticides that express the pesticide B.t. into interstate commerce;*

(6) *Grant such other relief as the Administrator deems just and proper*.[2]

For the reasons set forth below, EPA denies Petitioners' requests and determines that granting the relief requested by Petitioners is not required pursuant to the Agency's obligations under the Federal Insecticide, Fungicide, and Rodenticide Act ("FIFRA"). EPA has conducted a rigorous assessment of the *B.t.* plant-pesticides under FIFRA. Based on the relevant information and data currently available, these registrations do not cause unreasonable adverse effects. Further, EPA's actions in registering various *B.t.* plant-pesticides do not require a programmatic environmental impact statement under the National Environmental Policy Act, do not violate the requirements of the Administrative Procedure Act, and do not violate the Public Trust Doctrine.

All of the current registrations of *B.t.* plant-pesticides in cotton and corn plants will expire in 2001. EPA is comprehensively reassessing the expiring *B.t.* plant pesticide registrations and pest management resistance requirements, to ensure public health and environmental protection. This process will be scientifically-based and provide increased opportunities for public comment and participation on both EPA's scientific risk assessment and EPA's risk management proposals. The comprehensive reassessment will include (1) consideration by EPA of all currently available information on the risks and benefits of *B.t.* corn, cotton, and potato plant-pesticides; (2) development by EPA scientists of an updated risk assessment for *B.t.* plant-pesticides; (3) outside scientific peer review of EPA's risk assessment by the FIFRA Scientific Advisory Panel (SAP); (4) public comment on EPA's risk assessment; and (5) other opportunities for public involvement. EPA fully intends to reach a decision on the existing registrations in a timely fashion. This comprehensive reassessment will guide EPA in determining how it will handle future *B.t.* plant-pesticide applications for registration.

Moreover, we understand that the Federal Government intends to undertake an interagency assessment of environmental aspects of its regulation of biotechnology. A primary objective of such a assessment would be to identify any opportunities for strengthening the existing regulatory framework for assessing environmental risks associated with biotechnology. EPA will participate fully in this interagency assessment of Federal regulations applicable to biotechnology. To the extent that the

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[2] Petition at 33. For ease of reference, statements incorporated in this document that are directly attributable to Petitioners are italicized.

assessment results in recommendations to modify regulations, change data requirements, require additional research, or adopt other appropriate measures, EPA will factor such recommendations, as appropriate, into its reassessment of the *B.t.* plant-pesticides, and its regulation of plant-pesticides in general.

DISCUSSION

I. **Summary of Petition**

Petitioners make several broad arguments alleging that EPA has either violated or failed to comply with the requirements of several statutes. First, Petitioners argue that, pursuant to FIFRA, (1) the unreasonable adverse effects on the environment caused by the registration of *B.t.* endotoxins expressed in plants require cancellation of all such registrations and (2) that the adverse environmental effects of the registrations warrant initiation of a "Special Review" in accordance with the Agency's regulations at 40 C.F.R. Part 154.[3] Second, Petitioners argue that, pursuant to the National Environmental Policy Act (NEPA), EPA's registration of *B.t.* endotoxins expressed in plants require a programmatic environmental impact statement (PEIS).[4] Third, Petitioners assert that EPA's registration of *B.t.* endotoxins expressed in plants is arbitrary, capricious, and an abuse of discretion under the Administrative Procedure Act (APA).[5] Fourth, Petitioners assert that EPA's registration of *B.t.* endotoxins expressed in plants violates the Public Trust Doctrine.[6] EPA addresses each of these arguments, *seriatim*.

II. **EPA Response to Petitioners' Arguments**

A. Response to Petitioners' FIFRA Arguments

1. Currently Available Data and Information Support Continued Registration of *B.t.* Plant-Pesticides; Petitioners Do Not Provide Data or Information That Support Cancellation of *B.t.* Plant-Pesticide Registrations

a. Currently Available Data and Information Do Not Support Cancellation of the Challenged Registrations

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[3] Petition at 20-24.

[4] Petition at 24-27.

[5] Petition at 27-29.

[6] Petition at 30-32.

Petitioners assert that, pursuant to FIFRA, *"[t]he unreasonable adverse effects on the environment caused by the registration of genetically engineered plants expressing Bacillus thuringiensis requires cancellation of all [such] registrations."*[7] Petitioners are incorrect. First, as discussed comprehensively in this Response, the currently available evidence does not support the conclusion that the registered *B.t.* plant-pesticides may cause unreasonable adverse effects on the environment. Second, for products that EPA has reason to believe may result in adverse environmental effects in certain growing areas (as discussed below, non-high expression plants) over time, unless specific mitigation methods are employed, EPA has requested that the registrants of such products take specific mitigating methods for the 2000 growing season. If, after its comprehensive review of *B.t.* registrations, the Agency determines that these risks are real and such mitigating methods are necessary, EPA will only register such products in the future if these mitigation methods are incorporated as enforceable terms and conditions of such future registrations. Alternatively, EPA may take such other measures as may be necessary.

Pursuant to FIFRA, EPA regulates the development, sale, distribution, use, storage, and disposal of pesticides in interstate commerce. Section 3(a) of FIFRA provides that no person may distribute or sell to any person any pesticide that is not registered under FIFRA.[8] Section 3(c)(5) of FIFRA provides that EPA shall register a pesticide if presented with a registration application that demonstrates (1) the composition of the pesticide is such as to warrant the proposed claims for it; (2) the labeling and other material required to be submitted comply with the requirements of FIFRA; (3) it will perform its intended function without unreasonable adverse effects on the environment; and (4) when used in accordance with widespread and commonly recognized practice it will not generally cause unreasonable adverse effects[9] on the environment.[10] In addition, EPA may conditionally register

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7 Petition at 20.

8 7 U.S.C. § 136a(a). A comprehensive explication of the statutory scheme regulating pesticides under FIFRA and the Federal Food, Drug, and Cosmetic Act (FFDCA) is appended as Attachment A. EPA's pesticide regulations are set forth at 40 C.F.R. Parts 150-189.

9 FIFRA defines "unreasonable adverse effects on the environment" as "(1) any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide, or (2) a human dietary risk from residues that result from use of a pesticide in or on any food inconsistent with the standard under section 408 of the [FFDCA]." 7 U.S.C. § 136(bb).

10 7 U.S.C. 136a(c)(5). See Merrell v. Thomas, 807 F.2d 776, 781 (9th Circuit 1986) (the FIFRA registration standard "reflects the need to balance environmental and agricultural impacts").

pesticides under special circumstances as set forth at FIFRA Section 3(c)(7).[11] Once a pesticide has been registered, FIFRA Section 6 authorizes EPA to issue a notice of intent to cancel such registration if "it appears to the Administrator that a pesticide or its labeling or other material required to be submitted does not comply with the provisions of [FIFRA] or, when used in accordance with widespread and commonly recognized practice, generally causes unreasonable adverse effects" on the environment.[12] This provision has been interpreted to mean that the Administrator may cancel a pesticide registration upon a finding "that the pesticide commonly causes unreasonable risks."[13] Thus, because FIFRA Section 2 defines "adverse effects" as "unreasonable risks," EPA may initiate proceedings to cancel a pesticide registration under Section 6(b) if the Administrator determines that a pesticide "commonly creates a significant probability that [undesirable] consequences may occur."[14]

Petitioners assert that *"[t]he registration of [B.t.] plant-pesticides has created an unreasonable risk to the environment that outweighs any economic, social and environmental benefits, and as such the Administrator should withdraw all of the transgenic B.t. plant pesticide FIFRA registrations."*[15] Petitioners base this conclusion on assertions that plants expressing *B.t.* endotoxins will cause the evolution of *B.t.* resistant species, may create novel *B.t.* resistant weedy plant relatives, and may impact non-target beneficial organisms.[16]

Careful review of the data and studies cited by Petitioners in their arguments, and of the substantial amount of additional data that EPA has examined and analyzed in the normal conduct of its regulatory activities, does not support the conclusion that crops expressing registered *B.t.* plant-pesticides will potentially have such effects or pose an unreasonable risk that such effects may occur in the future. Moreover, EPA is aware of no data indicating that unreasonable adverse effects on the environment have occurred during the period that *B.t.* crops have been registered and used for commercial production (since 1995). Moreover, EPA has no reason to believe that such effects may occur during the continued duration of the current registrations.

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11 7 U.S.C. § 136a(c)(7).

12 7 U.S.C. 136d(b).

13 See, e.g., Ciba-Geigy v. United States Environmental Protection Agency, 874 F.2d 277, 279 (5th Cir. 1989).

14 Id.

15 Petition at 21.

16 Id.

b. Petitioners' arguments regarding development of pest resistance to *B.t.* endotoxins

In the Petition, Petitioners argue that the registered *B.t.* plant-pesticides will cause the evolution of *B.t.* resistant pest species.[17] Petitioners base this argument on the following assertions: (1) *EPA has been on notice since 1981 that resistance to B.t. has developed in certain pests*; (2) *transgenic plants producing B.t. endotoxins exert high selection pressure on pest populations because the B.t. plants maintain a constant killing dose throughout the growing season*; (3) *since the initial laboratory and field tests on transgenic plants expressing B.t. endotoxins, several common species of insect pests have evolved resistance to B.t. endotoxins*; (4) *a 1997 study found a higher than expected frequency of a resistance allele in tobacco budworm and that use of a 4% refuge*[18] *could lead to development of resistance in cotton bollworm and European corn borer in as little as 4 years*; (5) *a 1997 study strongly suggests that a resistance gene in the diamondback moth carries little genetic load, thus the resistance allele could have far higher frequencies in wild populations than previously predicted*; (6) *resistance to B.t. endotoxins worldwide in the diamondback moth has been found to be related to the extent of application of B.t.*; (7) *development of resistance to one of the Cry protein endotoxins often leads to cross-resistance to other Cry protein endotoxins*; (8) *the assumption that resistance to different strains of B.t. endotoxins requires separate autosomal mutations is incorrect*; (9) *polyphagous insects could result in cross-resistance developing far faster than previously believed*; (10) *contrary to conventional B.t. endotoxin preparations, plants expressing B.t. endotoxins have properties that make development of pest resistance more likely*.[19]

EPA will address the issue of pest resistance to *B.t.* plant-pesticides by first summarizing the Agency's current position on pest resistance management for registered *B.t.* plant-pesticides; second, EPA discusses the comprehensive and unprecedented efforts to assess and address the potential for development of resistance to *B.t.* plant-pesticides; third, EPA addresses Petitioners' specific arguments concerning the development and management of resistant insects.

c. Summary statement of EPA's current position on pest resistance management for registered *B.t.* endotoxins

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17 Id.

18 A refuge is an area that is untreated with a particular pesticide in order to leave portions of the pest population unexposed to that insecticide. For *B.t.* crops, a refuge is a stand of non-*B.t.* host plants that are managed to provide sufficient susceptible adult insects to mate with potential *B.t.*-resistant adult insects to dilute the frequency of resistance genes.

19 Petition at 11-15.

B.t. insect resistance management (IRM) is of great importance because of the threat insect resistance poses to the future use of *B.t.* pesticides. Public interest groups and organic farmers have expressed concern that the widespread planting of these genetically transformed plants will hasten the development of resistance to pesticidal *B.t.* endotoxins.

To address this real concern, EPA has imposed IRM data and monitoring requirements on registered *B.t.* plant-pesticides. Sound IRM will prolong the life of *B.t.* pesticides, and universal adherence to the plans is to the advantage of growers, producers, and researchers alike. EPA's strategy to address insect resistance is two-fold: (1) mitigate any significant potential for pest resistance development in the field by instituting IRM plans, and (2) continue to follow and act on the science of resistance management as it evolves.

Beginning with the first *B.t.* plant-pesticide registration, the Agency has taken steps to manage insect resistance to *B.t.* with IRM plans being an important part of the regulatory decision. These mitigation measures include IRM plans to prevent or manage resistance, field research and resistance monitoring, establishing refugia (a portion of the total acreage using non-*B.t.* seed), and implementation of appropriate changes in the plans as more information becomes available. It is believed that planting refugia will delay the development of insect resistance by maintaining insect susceptibility. EPA will continue to use science-based decision-making as it reevaluates IRM requirements for all *B.t.* crops.

Effective resistance management requires multiple tactics to decrease the selection pressure on target pests. For registered *B.t.* endotoxins, as with conventional pesticides, pest resistance management must be well integrated into the overall Integrated Pest Management (IPM) program. EPA believes that it has been demonstrated that the best way to reduce the selection pressure of foliar *B.t.* sprays is to minimize their use as much as possible.[20] Unique to plants expressing *B.t.* plant-pesticides, however, is the ability to produce continuous (season-long) expression at relatively high doses (as compared to other insecticides). To minimize selection pressure, and thus resistance, the scientific community supports the use of a high dose coupled to a structured refuge as the most feasible insect resistance management strategy for *B.t.* crops. In addition, appropriate scouting, resistance monitoring for alterations in pest susceptibility, adoption of good IPM practices, and education are critical to the success of any resistance management strategy.

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[20] See, e.g., Roush, R. T. and Tingey, W. M., Strategies for management of insect resistance to synthetic and microbial insecticides, in Advances in Potato Pest Biology and Management 237 (American Phytopathological Society Press, St. Paul, MN 1994); Tabashnik, B. E., Evolution of resistance to *Bacillus thuringiensis*, 39 Ann. Review of Entomology 47 (1994); Roush, R. T., Managing pests and their resistance to *Bacillus thuringiensis*: Can crops be better than sprays?, 4 Biocontrol Science and Technology 501 (1994).

Using high dose expression coupled to structured refuges for delaying pest resistance to *B. thuringiensis* is the most promising strategy to manage insect resistance.[21] The high dose/structured refuge strategy has been widely endorsed by the scientific community. See, for example, the reports of multiple SAP subpanels, EPA's White Paper, USDA NC-205 (Research and Extension Entomologists of the North Central Regional Research Project (NC-205), Ecology and Management of European Corn Borer and Other Stalk Boring Lepidoptera), International Life Sciences Institute (ILSI), Hardee *et al.*, and the Union of Concerned Scientists'(UCS) "Now or Never" Report.[22] EPA has implemented appropriate IRM plans based on the high dose/structured refuge strategy of resistance management as recommended by various scientific expert groups. EPA continually reevaluates whether these plans are adequate, as new scientific information becomes available.

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21 Liu, Y.B. and Tabashnik, B.E., Experimental evidence that refuges delay insect adaptation to *Bacillus thuringiensis*, 264 Proc. R. Soc. Lond. B. 605 (1997); Alstad, D.N. and Andow, D.A., Managing the evolution of insect resistance to transgenic plants, 268 Science 1894 (1995); Gould, F., Evolutionary biology and genetically engineered crops, 38 Biosci. 26 (1998); Mallet, J. and Porter, P., Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? 255 Proc. R. Soc. Lond. B. 65; (1992); McGaughey, W.H. and Whalon, M.E., Managing insect resistance to *Bacillus thuringiensis* toxins, 258 Science 1451 (1992); Tabashnik, B.E., Evolution of resistance to *Bacillus thuringiensis*, 39 Ann. Rev. Entomol. 47 (1994); Tabashnik, B.E., Delaying insect adaptation to transgenic plants: seed mixtures and refugia reconsidered, 255 Proc. R. Soc. Lond. B. 7 (1994).

22 U.S. EPA Scientific Advisory Panel on *Bacillus thuringiensis* (Bt) Plant- Pesticides, February 9-10,1998 (Docket Number: OPPTS-00231); U.S. Environmental Protection Agency, The Environmental Protection Agency's White paper on *B.t.* Plant-Pesticide Resistance Management (EPA Publication 739-S-98-001); Ostlie, K. R., W. D. Hutchinson, and R. L. Hellmich, *B.t.*-Corn & European Corn Borer: Long-Term Success Through Resistance Management, North Central Regional Extension Publication NCR 602 (1997); NC-205 Supplemental Report, Supplement to: *B.t.* corn & European corn borer: long-term success through resistance management, NCR Publication 602 (1998); International Life Sciences Institute, An evaluation of insect resistance management in Bt field corn: a science-based framework for risk assessment and risk management, Report of an expert panel (1999); Hardee, D.D., J.W. Van Duyn, M.B. Layton, and R.D. Bagwell, Bt cotton for management of tobacco budworm and bollworm: Continued effectiveness by managing resistance (Hardee, D.D. and J.W. Van Duyn, eds., in press); Mellon, M. and J. Rissler, Now or never: Serious new plans to save a natural pest control, (M. Mellon and J. Rissler, eds., 1999).

Refuges of non-*B.t.* crops enable survival of susceptible individuals, which decreases the intensity of selection and slows evolution of resistance.[23] Under ideal conditions, relatively large numbers of susceptible individuals from refuges survive and mate with few resistant survivors from treated areas. Therefore, a high dose coupled to a structured refuge will effectively dilute resistance in the insect population. High dose expression of *B.t.* toxin in plants will kill all but rare homozygous recessive resistant individuals. It is believed that nearly all susceptible individuals will be killed by the high dose. Moreover, coupling high dose expression with structured non-*B.t.* refuges allows survival of susceptible individuals, and maximizes the probability that rare resistant homozygotes will mate with susceptible individuals, producing progeny that are susceptible to the *B.t.* crop. Thus, over time, the presumed low levels of initial resistance are effectively diluted. The efficacy of high dose coupled to a structured refuge in mitigating resistance development has been demonstrated by projections from computer simulations, data from small-scale experiments, and several years of commercial use. These suggest that, in theory, if resistance is recessive and mating is random, refuges can greatly delay insect adaptation to *B. thuringiensis*.[24]

While a high dose is preferable for all target pests, not all registered *B.t.* endotoxins are expressed in transformed plants at high doses.[25] Effective insect resistance management is still possible

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[23] Shelton, A.M., J.D. Tang, R.T. Roush, T.D. Metz, and E.D. Earle, Field tests on managing resistance to *B.t.*-engineered plants, 18 Nature Biotechnology 339 (2000).

[24] Alstad, D.N. and Andow, D.A., Managing the evolution of insect resistance to transgenic plants, 268 Science 1894 (1995); Gould, F., Evolutionary biology and genetically engineered crops, 38 Biosci. 26 (1988); Mallet, J. and Porter, P., Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy?, 255 Proc. R. Soc. Lond. B. 165 (1992); McGaughey, W.H. and Whalon, M.E., Managing insect resistance to *Bacillus thuringiensis* toxins, 258 Science 1451 (1992); Tabashnik, B.E., Evolution of resistance to *Bacillus thuringiensis*, 39 Ann. Rev. Entomol. 47 (1994); Tabashnik, B.E., Delaying insect adaptation to transgenic plants: seed mixtures and refugia reconsidered 255 Proc. R. Soc. Lond. B. 7 (1994).

[25] Examples of *B.t.* crops that do not express *B.t.* endotoxin at high levels and, thus, do not provide a high dose to particular target pests are Dekalb's Cry1Ac corn and Mycogen's and Novartis' Event 176 Cry1Ab corn. (An "event" denotes the successful transformation of a crop plant by specific insertion of the genetic material in a specific crop plant that led to the commercialized *B.t.* crop hybrid. Some manufacturers refer to specific product lines by event number). For the 2000 growing season, Dekalb, Mycogen, and Novartis have agreed to specific mitigation measures to address the Agency's concerns regarding IRM with respect to such non-high dose products. Pending the Agency's comprehensive public review process of all existing *B.t.* registrations, these measures, or others, as may be appropriate, may be specifically required as terms and conditions of any future registrations of such non-high dose

even if the transformed plant doesn't express the *B.t.* endotoxin at a high dose.[26] If the transformed plant doesn't express the *B.t.* endotoxin at a high dose, the IRM plan should include significantly increased refuge size, increased scouting and monitoring, and/or prohibition of sales of the non-high dose product in certain areas.

EPA believes it is significant that, after four years of full-scale commercialization of *B.t.* crops for which the Agency currently has data (1996, 1997, 1998, and 1999), with approximately 17 million total acres of *B.t.* corn, *B.t.* potato, and *B.t.* cotton planted in 1998, EPA has received no confirmed evidence that field resistance to any *B.t.* endotoxin expressed in these crops has occurred in any insect species. As part of the mandatory terms and conditions of the *B.t.* plant-pesticide registrations, registrants are required to submit monitoring data on the susceptibility of field-collected insect pests to various *B.t.* proteins. No effects, outside the normal ranges of susceptibility to the various *B.t.* proteins, have been reported for the tobacco budworm, pink bollworm, or European corn borer. In addition, there have been no reports of changes in susceptibility for the Colorado potato beetle (CPB, *Leptinotarsa decemlineata* (Say)). The cotton bollworm (also known as the corn earworm), however, has a natural tolerance to the Cry1Ac toxin. Some degree of increased (about 10-fold) tolerance (not resistance) to the Cry1Ac toxin found in *B.t.* cotton in CBW populations from South Alabama, the Mississippi Delta, Georgia, the Florida Panhandle, and South Carolina has been reported based on laboratory bioassays during the three-year period from 1996 to 1998.[27] But, increased tolerance should not be interpreted as resistance.[28] There is no evidence of field failure of *B.t.* cotton due to either TBW or CBW resistance. These results, however, do indicate that factors selecting for CBW resistance may already be increasing in the field and that continued monitoring and further analysis is necessary. The registrant is investigating these reports further and the Agency will continue its close scrutiny regarding the susceptibility of CBW to the Cry1Ac protein. The Agency is prepared

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products. Or, EPA may take such other measures as may be necessary.

26 Gould, F., Sustainability of insecticidal cultivars: integrating pest genetics and ecology, 43 Ann. Rev. Entomol. 701, 719 (1998) ("If we are to use TICs [transgenic insecticidal crops] with moderate expression in a sustainable manner, refuge size must be significantly increased.").

27 Sumerford, D.V., D.D. Hardee, L.C. Adams, and W.L. Solomon, Status of Monitoring for Tolerance to Cry1Ac in Populations of *Helicoverpa zea* and *Heliothis virescens*: Three-Year Summary, 1999 Proceeding of the Beltwide Cotton Conferences (1999).

28 As used here, "tolerance" is defined as a change in susceptibility that has not been determined to be related to the evolution of resistance. Resistance occurs through a process of selection, whereby a population becomes less susceptible to the pesticide. Most entomologists state that actual "resistance" occurs only after there is a 10X difference in the LC_{50} of susceptible and resistant individuals, respectively. If there is a less than 10-fold difference in the LC_{50} of susceptible and resistant individuals, it is referred to as tolerance.

to take appropriate regulatory action, if warranted, as further resistance monitoring data become available.

d. EPA's ongoing efforts to assess and address the potential development of pest resistance to *B.t.* endotoxins

(i) Ongoing development of IRM plans

In registering *B.t.* plant-pesticides, EPA has taken extensive and unprecedented measures to significantly reduce the likelihood that insects exposed to *B.t.* plant-pesticides will develop resistance. As a result of numerous public meetings and consultations with experts in the field, EPA has determined that good insect resistance management should utilize a high dose/structured refuge strategy.[29] EPA required that all applicants for registration of *B.t.* plant-pesticides provide the Agency with insect resistance management (IRM) plans. Moreover, EPA has mandated certain risk mitigation measures to ensure that selection pressure is effectively managed and the risk of insect resistance development to *B.t.* plant-pesticides is minimized. The Agency has required or recommended generation of specific research data, development and implementation of structured refuges, annual resistance monitoring, remedial action plans, grower education, and sales and research reporting for certain *B.t.* crops as part of the development and implementation of long-term IRM strategies. EPA believes that the long-term IRM strategies that have been developed through an extensive public consultative process sufficiently mitigate the development of insect resistance to *B.t.* plant-pesticides.[30]

Well before registration of the first *B.t.* plant-pesticide in 1995, EPA engaged in consultations regarding resistance management for *B.t.* plant-pesticides at EPA FIFRA Scientific Advisory Panel (SAP)[31] meetings attended by EPA, USDA, potential registrants, academics, and public interest groups. In addition, potential registration applicants had been conducting or sponsoring research on the

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29 As discussed *infra*, the current scientific data and information support the conclusion that non-high dose products require additional pest management practices, which have been implemented.

30 EPA's reviews of the resistance management strategies for registered *B.t.* plant-pesticides are summarized in the pesticide Fact sheets available at: "http:\\www.epa.gov\pesticides\biopesticides" and individual regulatory decision memoranda. The Fact Sheets and individual regulatory decision memoranda have been placed in the Special Docket for this action.

31 The Office of Pesticide Programs FIFRA Science Advisory Panel is composed of non-Agency scientists who perform peer reviews of the Agency's scientific risk assessments and guidelines for pesticide regulation. The various SAP and other scientific meetings that EPA has convened to obtain expert scientific input on *B.t.* crop matters are discussed in Appendix B.

biology and ecology of affected insects and crops (e.g., adult and larval movement, ovipositional and mating behavior, population dynamics, cross-resistance potential, potential resistance mechanisms, refuge strategies, susceptibility, etc.) to better understand long-term resistance management of *B.t.* crops to slow or halt the development of insect resistance. Good insect resistance management is dependent on multiple tactics to decrease the selection pressure on the target pest(s) and employment of different mortality sources. The 1995 SAP subpanel on plant-pesticides agreed with EPA on the essential elements of an insect resistance management plan: (1) knowledge of pest biology and ecology, (2) appropriate dose expression strategy, (3) appropriate refuges, (4) monitoring and reporting of incidents of pesticide resistance development, (5) employment of integrated pest management, (6) communication and educational strategies on use of the product and (7) development of alternative modes of action.[32]

Subsequent to registration of the first *B.t.* crops in 1995, substantial information has been developed that enhances the Agency's understanding of the requirements of IRM plans. EPA convened three SAP subpanels (in 1992, 1995, and 1998); two public hearings in March and May 1997; and two Pesticide Program Dialogue Committee (PPDC) meetings in July 1996 and January 1999 to address, in part, IRM for plants expressing *B.t.* endotoxins.[33] USDA sponsored a *B.t.* crop IRM forum in April 1996. As part of its scientific basis for developing IRM recommendations and requirements for *B.t.* crops, EPA relied on other scientific expert group reports including: the USDA North Central Regional Research Committee NC-205 (NC-205) refuge recommendations, a report by the International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI), and

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32 Science Advisory Panel, Subpanel on Plant-Pesticides, March 1, 1995. The 1995 SAP considered a set of scientific issues in connection with Monsanto Company's application for registration of a plant-pesticide containing the active ingredient *Bacillus thuringiensis* subsp. *tenebrionis* delta-endotoxins. The final report of the 1995 SAP panel is at Docket Number: OPP-00401, and is in the Special Docket for this Response. Final reports of all SAP meetings related to *B.t.* crops are available at http://www.epa.pesticides/. Other SAP/Biotechnology Science Advisory Panel (BSAC) Meetings were held by EPA on other issues related to plant-pesticides. See Appendix B.

33 The Pesticide Program Dialogue Committee (PPDC) is composed of representatives of different stakeholder organizations who provide the Agency input on new and ongoing policy developments related to pesticide regulations. The minutes of the PPDC and the final reports of the SAP meetings are available at http://www.epa.pesticides/ppdc . The final reports of the relevant SAP meetings are in the Special Docket for this Response. The 1995 SAP final report is at Docket Number: OPP-00401. The 1998 SAP final report is at Docket Number: OPPTS-00231. The March and May 1997 public hearings are summarized in the EPA "White Paper" on IRM: The Environmental Protection Agency's White Paper on *Bacillus thuringiensis* Plant-pesticide Resistance Management (January 14, 1998) [EPA Publication 739-S-98-001].

a publication by the Union of Concerned Scientists.[34] EPA also presented its own analysis of *B.t.* plant-pesticide resistance management in a January 1998 paper.[35] The White Paper was the focal point of discussion at the February, 1998 SAP subpanel meeting on IRM for *B.t.* crops. The 1998 SAP subpanel recommended that EPA require the use of structured refuges in all registrations of plants expressing *B.t.* endotoxins, unless it can be shown conclusively that such refuges would harm, rather than aid, durability of the resistance management plan.[36] The subpanel indicated that acceptable refuge configurations may vary among regions but that a structured refuge should provide sufficient susceptible adult insects to mate with potential *B.t.*-resistant adult insects to dilute the frequency of resistance genes.

While the referenced reports, symposia, and meetings support the consensus understanding that a high dose/structured refuge strategy is ideal for mitigating insect resistance to *B.t.* plant-pesticides, EPA continues to investigate IRM to determine the appropriate content and means of implementation of IRM plans. As a result of continuing concerns about the potential development of insect resistance to *B.t.*, and to ensure that *B.t.* products remain effective for all farmers, a refuge must be established on a per farm basis. The issue of refuge size and deployment continues to be investigated extensively by industry, academia, consumer advocates, and the federal government. EPA is working with USDA, academia, growers, registrants, and public interest groups to ensure that new genetically modified pesticide products can be used without the development of pest resistance. EPA and USDA held two public workshops in 1999 on IRM plans for *B.t.* crops.[37] Academics, growers, industry, and public interest groups discussed the current and future refuge strategies, grower education, compliance concerns, resistance monitoring, and other issues related to IRM for *B.t.* crops. EPA/USDA published a joint position paper on *B.t.* crop insect resistance management that was used as a basis for discussion

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34 Ostlie, K. R., W. D. Hutchinson, and R. L. Hellmich (eds.), *Bt*-Corn & European Corn Borer: Long-Term Success Through Resistance Management, North Central Regional Extension Publication NCR 602 (1997); NC-205 Supplemental Report, Supplement to: *Bt* corn & European corn borer: long-term success through resistance management, NCR Publication 602 (1998); International Life Sciences Institute (ILSI), An evaluation of insect resistance management in *Bt* field corn: a science-based framework for risk assessment and risk management; Report of an expert panel (1999); Mellon, M. and J. Rissler [eds.], Now or never: Serious new plans to save a natural pest control, Union of Concerned Scientists (1998).

35 The Environmental Protection Agency's White Paper on *B.t.* Plant-pesticide Resistance Management, EPA 739-S-98-001 (1998).

36 Scientific Advisory Panel, on *Bacillus thuringiensis* (Bt) Plant- Pesticides, February 9-10, 1998 (Docket Number: OPPTS-00231). SAP final reports are also available at http://www.epa.gov/pesticides/ and in the Special Docket for this action.

37 June 18, 1999, Chicago, IL and August 26, 1999, Memphis, TN.

at both the *B.t.* corn and the *B.t.* cotton IRM workshops in June and August 1999, respectively.[38] EPA continues to work closely with public interest groups, the agricultural community, academia, and industry to ensure that appropriate IRM plans and grower education programs are in place for the 2000 growing season and beyond. As discussed above, EPA will conduct a scientifically based public process in 2000 to reevaluate the current IRM plans.

(ii) The consensus scientific opinion is that high-dose expression and refuges are an important component of a *B.t.* plant-pesticide IRM strategy

The current consensus scientific opinion is that a structured refuge/high dose strategy is the most feasible way of mitigating the rate of resistance development at this time for *B.t.* crops. Other resistance management approaches have been discussed, but, at present, they have not gained acceptance or are more difficult to implement with today's technology.[39] Refuges are stands of non-*B.t.* host plants that are managed to provide sufficient susceptible adult insects to mate with potential *B.t.*-resistant adult insects to dilute the frequency of resistance genes. The 1998 SAP subpanel on *B.t.* crop resistance management defined an operational high dose as 25 times the amount of *B.t.* delta-endotoxin necessary to kill susceptible individuals. The 1998 SAP subpanel also suggested that 500 susceptible adults in the refuge should be available for mating with every potentially resistant adult in a *B.t.* field (assuming a resistance allele frequency of 5×10^{-2}).[40] Refuge options should be developed and managed to achieve this goal. The placement and size of the structured refuge should be based on the existing target pest biology data (e.g., larval and adult movement, mating and ovipositional behavior, fecundity). The refuge should be planted with an agronomically similar hybrid and as close as possible to, and at the same time as, the *B.t.* crop. For crops that do not express a high dose of a *B.t.* plant-pesticide, the refuge size should be increased or alternative control measures should be utilized.[41]

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38 The EPA/USDA position paper and workshop proceedings are found in the Special Docket and at http://www.epa.gov/pesticides/biopesticides (see "Related Documents").

39 Gould, F., Sustainability of insecticidal cultivars: integrating pest genetics and ecology, 43 Ann. Rev. Entomol. 701 (1998); Roush, R. T., Can we slow adaptation by pests to insect resistant crops?, pp. 242, in G. J. Persley [ed.], Biotechnology and Integrated Pest Management. CAB Int., Oxon, UK (1996).

40 Scientific Advisory Panel, on *Bacillus thuringiensis* (Bt) Plant-Pesticides, February 9-10, 1998 (Docket Number: OPPTS-00231) (available in the Special Docket for this Response).

41 Gould, F., Sustainability of insecticidal cultivars: integrating pest genetics and ecology, 43 Ann. Rev. Entomol. 701 (1998).

In the development of refuge options, the following considerations should be made. First, the applicability of an in-field versus external refuge for each target (susceptible) pest should be evaluated based on larval and adult movement, ovipositional and mating behavior. The proximity of an external refuge must be within the normal adult flight range to mating/ovipositional habitats. If there are multiple pests, the structured refuge options should be inclusive of all potential target (susceptible) pests. If there are regional pest considerations, the structured refuge options should reflect these considerations. Therefore, structured refuge options should be designed within the feasibility and current understanding of the technology to address all of the target pest species including the pest with the greatest susceptibility to the *B.t.* toxin. Second, structured refuge options should be designed with a high degree of grower adoption in mind. Consideration should be made if there is a single dominant pest for a particular region or locality and whether there is a high dose for that pest. While a high dose is preferable for all target pests, not all registered *B.t.* endotoxins are expressed in transformed plants at high doses.[42] Effective insect resistance management is still possible even if the transformed plant doesn't express the *B.t.* endotoxin at a high dose.[43] If the transformed plant doesn't express the *B.t.* endotoxin at a high dose, the IRM plan should include significantly increased refuge size, increased scouting and monitoring, and/or prohibition of sales of the non-high dose product in certain areas. In addition, refuge strategies should consider whether there are stacked genes versus single genes to control the target pests.[44] If alternative crop hosts are to be used as a refuge, justification needs to be provided as to the applicability of these alternative hosts as effective refuges for each target pest. Resistance management strategies, including structured refuge options, should be flexible to accommodate rapidly changing technology (e.g., gene stacking or adding additional *B.t.* proteins), current research data, and improved understanding of resistance management. EPA is working closely with academia, other federal agencies, public interest groups, industry, and growers to develop and

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42 Examples of *B.t.* crops that do not express *B.t.* endotoxin at high levels and, thus, do not provide a high dose to particular target pests are Dekalb's Cry1Ac (DBT-418) corn and Mycogen's and Novartis' Event 176 Cry1Ab corn. For the 2000 growing season, Dekalb, Mycogen, and Novartis have agreed to specific mitigation measures to address the Agency's concerns regarding IRM with respect to non-high dose products. Pending the Agency's comprehensive public review process of all existing *B.t.* registrations, these measures, or others, as may be appropriate, may be specifically required as terms and conditions of any future registrations of such non-high dose products. Or, EPA may take such other measures as may be necessary.

43 Gould, F., <u>Sustainability of insecticidal cultivars: integrating pest genetics and ecology</u>, 43 Ann. Rev. Entomol. 701, 719 (1998) ("If we are to use TICs [transgenic insecticidal crops] with moderate expression in a sustainable manner, refuge size must be significantly increased.").

44 "Stacked genes" occur when more than one gene is introduced into a single crop plant variety, e.g., the presence of two genes for two different *B.t.* endotoxins targeting the same pest spectrum.

implement effective insect resistance plans, based on the current science, that are consistent, effective, and flexible to address regional pest management concerns.

To summarize, currently available data and information support the following best management principles for use as guidance in developing IRM strategies for plants expressing *B.t.* endotoxins.[45]

(1) A specific IRM plan is ideal to ensure long-term resistance management.[46]

(2) A high dose/structured refuge strategy is ideal to ensure long-term resistance management. If a transformed plant expresses a non-high dose rather than a high dose for a particular target pest, the refuge size should be increased to balance the lower dose. Wild hosts and other crops might serve as part of a larger refuge, but data must support the contribution of these hosts to overall pest population size in different geographic areas. Spatial requirements for non-high dose *B.t.* crops might not be as strict as they are for high dose *B.t.* crops.[47]

(3) Grower education, adoption, and compliance are essential to the implementation and success of a long-term resistance management strategy.

(4) *B.t.* crops are to be used as part of an integrated pest management program to enhance pest management goals.

(5) Coordinated annual performance monitoring and surveillance is necessary to detect or follow resistance development.

(6) Immediate and coordinated remedial action for suspected and confirmed incidents of resistance is necessary.

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[45] EPA's best management principles are based on the recommendations of the 1998 SAP subpanel on *B.t.* plant-pesticide resistance management, and existing scientific literature.

[46] The 1995 SAP subpanel on plant-pesticides stated that the essential elements of an IRM plan are: (1) knowledge of pest biology and ecology, (2) appropriate dose expression strategy, (3) appropriate refuges (primarily for insecticides), (4) monitoring and reporting of incidents of pesticide resistance development, (5) employment of IPM, (6) communi-cation and educational strategies on use of the product and (7) development of alternative modes of action. Docket Number: OPP-00401.

[47] Gould, F., <u>Sustainability of insecticidal cultivars: integrating pest genetics and ecology</u>, 43 Ann. Rev. Entomol. 701, 719 (1998).

(7) IRM strategies should be tailored to address specific regional resistance management concerns, as appropriate.

(8) Deployment of IPM tactics with different modes of action, including conventional pesticides, *B.t.* toxins expressed in crops with different modes of action, biological control methods, and other control methods, is essential for sustainable pest management goals.

(9) Continued resistance management research should be conducted to evaluate the effectiveness of, and be used to modify, as necessary, IRM strategies for *B.t.* crops.

(iii). Current IRM Requirements of Registered *B.t.* crops

There are currently ten separate registered products containing *B.t.* plant-pesticides expressed in potato, corn (field corn, popcorn, and sweet corn), and cotton.[48] While EPA has not formally published a generally applicable IRM policy or specific IRM data requirements for plants expressing registered *B.t.* plant-pesticides, each of the currently registered *B.t.* crops is subject to specific IRM requirements. *B.t.* corn and *B.t.* cotton also have data generation and resistance monitoring requirements. Insect resistance management is a dynamic process. The Agency has adjusted the IRM requirements as more scientific data have become available. A brief description of the IRM requirements for each of the registered *B.t.* crops follows.

B.t. potato (Cry3A). EPA initially established voluntary IRM recommendations, consistent with the 1995 SAP subpanel report.[49] In May 1999, consistent with the 1998 SAP subpanel report,[50] EPA imposed a mandatory 20% structured refuge of non-*B.t.* potatoes to be planted in close proximity to the *B.t.* potato field to mitigate the development of Colorado potato beetle (CPB) resistance. Thus, the previously voluntary 20% refuge for this registration became a mandatory term and condition of the registration. Since 1997, Monsanto/NatureMark has required that growers plant this refuge as part of the terms of a grower contract. Monsanto/NatureMark surveys all *B.t.* potato growers annually for compliance with and understanding of the 20% refuge. Results of annual surveys provided by Monsanto/NatureMark indicate an extremely high level (virtually 100%) of grower compliance. Monsanto indicates that they would refuse to renew the grower licence for recalcitrant growers who fail

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48 Current *B.t.* registrations are set forth in a tabular format in Appendix C.

49 Science Advisory Panel, Subpanel on Plant-Pesticides, March 1, 1995 (Docket Number: OPP-00401).

50 Scientific Advisory Panel on *Bacillus thuringiensis* (Bt) Plant-Pesticides, February 9-10, 1998 (Docket Number: OPPTS-00231).

to comply with the refuge requirements in the grower contract. Prior to this, Monsanto would intensify its grower education programs on an individual and regional level if compliance becomes a concern.[51]

In 1995, consistent with the SAP subpanel's report, EPA recommended that a detailed resistance monitoring/surveillance program and a remedial action plan be developed and instituted for *B.t.* potato. Monsanto/NatureMark provided the Agency with a summary of the baseline susceptibility work, development of a discriminating dose assay, and a detailed monitoring/surveillance program description including an appropriate remedial program.[52] The resistance monitoring plan included sampling sites, grower education on resistance sampling, collection of specimens to evaluate for resistance, and specific recommendations on eradication of resistant individuals to prevent survival of a resistant population. Naturemark developed a discriminating dose assay for CPB. Naturemark has a toll-free number for growers to report unusual CPB survival or for other technical information. Naturemark also developed and distributed user guidelines explaining the size and deployment of refuges and resistance monitoring. As part of the resistance monitoring program, Naturemark established an outreach program in cooperation with seed suppliers and extension entomologists to look for unexpected levels of CPB survival, and developed a rapid serological test to identify plants containing the Cry3A protein.[53] Monsanto/Naturemark has revised the Cry3A potato user guides each year based on new scientific data.

The Monsanto/Naturemark remedial action plan instructs customers to contact them (e.g., using a toll-free customer service number) if incidents of unexpected levels of CPB damage occur. If CPB are found to be resistant to *B.t.*, they can be treated immediately with a conventional insecticide to prevent further reproduction and movement. Naturemark reports in its 1996-1997 status report of resistant management activities that there were 2 situations in which growers alerted Naturemark of CPB larvae surviving on NewLeaf plants. In both cases, due to planting errors, the plantings were non *B.t.*-expressing plants rather than NewLeaf plants. Naturemark indicates that they confirmed that the plants did not contain the Cry3A gene. There were no reports of problems for the 1998 and 1999 growing season reported to the Agency.

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51 IRM compliance issues will be part of the Agency's comprehensive review of *B.t.* plant-pesticide registrations. EPA will consider whether to make such compliance measures mandatory terms and conditions of subsequent registrations.

52 The baseline susceptibility work (1992-1996) for CPB populations to the Cry3A protein used a total of 79 geographically distinct populations from commercial potato farms in 15 states and 2 provinces of Canada that were assayed for susceptibility.

53 See EPA White Paper for summary of the IRM plan for *B.t.* potato. (EPA Publication 739-S-98-001, docket number OPPTS-00231).

<u>*B.t.* cotton (Cry1Ac).</u> Since 1995, EPA has required two structured refuge options for *B.t.* cotton to mitigate the development of resistance in tobacco budworm (TBW, *Heliothis virescens* (Fabricius)), cotton bollworm (CBW, *Helicoverpa zea* (Boddie)), and pink bollworm (PBW, *Pectinophora gossypiella* (Dyar)). In *B.t.* cotton, there is a high dose for TBW and PBW and a moderate dose for CBW.[54] To decrease the potential for target insect pests to become resistant to Cry1Ac, two specific refuge options were mandated as terms and conditions of *B.t.* cotton registrations. "Option A: For every 100 acres of cotton with the Bollgard gene planted, plant 25 acres of cotton without the Bollgard gene that can be treated with insecticides (other than foliar *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*) products) that control the tobacco budworm, cotton bollworm, and pink bollworm. Option B: For every 100 acres of cotton with the Bollgard gene planted, plant 4 acres of cotton without the Bollgard gene that cannot be treated with acephate, amitraz, endosulfan, methomyl, profenofos, sulprofos, synthetic pyrethroids, and/or *B.t.k* insecticides labeled for the control of tobacco budworm, cotton bollworm, and pink bollworm. The refuge acreage must be managed similarly to Bollgard cotton."[55]

In addition, if cotton with the Bollgard gene exceeds 75% of the total amount of the cotton planted in any single county or parish in any year, growers in that county or parish choosing to use the 4% untreated refuge option the following year will be required to plant the 4% refuge within one mile of the respective Bollgard cotton field. Similarly, if EPA grants a registration for cotton containing the *B.t.k*. insect control protein to another company, EPA will determine whether the combined acreage of cotton containing the *B.t.k* insect control protein exceeds 75% of the total amount of the cotton planted in a single county or parish and inform the registrants that the 4% refuge must be planted in a single county or parish and inform the registrants that the 4% refuge must be planted within one mile of the respective Bollgard cotton or other B.t.k cotton fields.

As a condition of the October 31, 1995 registration, EPA required Monsanto to submit a plan for a workable resistance monitoring program and existing TBW, CBW, and PBW susceptibility data by March 1, 1996. A preliminary resistance monitoring report must be submitted to EPA annually by November 1 and a final report submitted by January 31. EPA reviewed the resistance monitoring plan submitted in March 1996 and found it to be generally acceptable. The 1998 SAP subpanel concurred in this assessment. The resistance monitoring programs examine changes in the baseline susceptibility of

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54 Scientific Advisory Panel on *Bacillus thuringiensis* (Bt) Plant- Pesticides, February 9-10, 1998 (Docket Number: OPPTS-00231).

55 EPA recently became aware that a registrant has altered its grower guide to instruct growers that it is acceptable to use acephate on the putatively unsprayed refuge, if the insecticide is used at a low concentration (purportedly a concentration low enough to be ineffective against TBW, CBW, and PBW - but effective against other pests). EPA did not approve this revision of the grower guide and has directed the registrant not to give growers this erroneous and unapproved guidance.

TBW, PBW, and CBW populations (regional level) to Cry1Ac. EPA also required a remedial action plan if there were either suspected or confirmed incidents of insect resistance. Monsanto is required to instruct customers to contact the company regarding unexpected levels of TBW, CBW, or PBW damage or if resistance is suspected. Monsanto is to investigate and identify the cause of such damage. Based on these investigations, appropriate remedial action is required to mitigate resistance. Resistance monitoring will be intensified in instances of suspected or confirmed resistance. Any confirmed incidents of resistance are required to be reported to the EPA under the terms and conditions of the registration as well as under FIFRA section 6(a)(2). Monsanto has instructed its customers to have regular surveillance programs and report any unexpected levels of TBW, CBW, and PBW damage to them and to their local extension agents. Remedial actions include: inform customers and extension agents in the affected areas of resistance problems, implementing alternative means to reduce or control the resistant populations, increasing monitoring in the affected areas, modifying refuges in the affected areas, and ceasing sales in the affected and bordering counties. Industry cooperation with extension and academic entomologists and consultants is considered important in communicating specific information of definitions of "unexpected damage" and appropriate remedial action.

As noted above, Monsanto is required by the terms and conditions of the *B.t.* cotton registration to monitor the susceptibility of TBW, CBW, and PBW to the Cry1Ac toxin and submit annual resistance monitoring reports to the Agency. Monsanto has submitted the results of its resistance monitoring programs for the 1996-1998 growing seasons. These results indicate no evidence of TBW, CBW, or PBW resistance to the Cry1Ac toxin in the field.[56] Resistance monitoring results for the 1999 growing season were due January 31, 2000. Monsanto requested an extension for submission of 1999 resistance monitoring data until April 1, 2000. EPA granted the requested extension.

Monsanto reported to EPA suspected incidents of bollworm resistance to *B.t.* cotton in July 1996. Upon further investigation, available scientific information indicated that TBW and CBW susceptibilities to the Cry1Ac were unchanged in the affected locations after the 1996 cotton growing season. The Cry1Ac expression in the *B.t.* cotton was as expected. There was no evidence for TBW or CBW resistance to the Cry1Ac toxin. Thus, the reports of suspected resistance were unconfirmed.

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[56] EPA's 1998 "White Paper;" Simmons, A.L., T. J. Dennehy, B.E. Tabashnik, L. Antilla, A. Bartlett, D. Gouge, R. Staten, Evaluation of *B.t.* Cotton Deployment Strategies and Efficacy Against Pink Bollworm in Arizona, in Proceedings of the Beltwide Cotton Conference, at. 1025-1030 (1998); Sumerford, D.V., D.D. Hardee, L.C. Adams, and W.L. Solomon, Status of Monitoring for Tolerance to Cry1Ac in Populations of *Helicoverpa zea* and *Heliothis virescens*: Three-Year Summary, 1999 Proceeding of the Beltwide Cotton Conferences (1999); Patin, A.L., T.J. Dennehy, M.A. Sims, B.E. Tabashnik, Y-B. Liu, L. Antilla, D. Gouge, T. J. Henneberry, R. Staten, Status of pink bollworm susceptibility to *Bt* in Arizona, in Proceedings of the Beltwide Cotton Conferences (1999).

In 1996, Monsanto investigated widespread claims of *B.t.* cotton failure in the Brazos River Bottoms in East Texas and reported this information to the EPA immediately in July 1996. The concern was whether the loss in efficacy was due to CBW resistance to the Cry1Ac delta endotoxin. Monsanto investigated the cause of these "failures" at the affected sites. CBW and *B.t.* cotton tissue was collected from high infestation areas to investigate claims of CBW resistance. CBW susceptibility and *B.t.* expression in *B.t.* cotton areas affected by high bollworm infestations were determined. There was no change in bollworm susceptibility and in *B.t.* expression in these areas as compared to other locations. These studies showed no detectable level of resistance in these populations. Experts agree that the *B.t.* cotton performed as expected under high infestation conditions of CBW. Reports indicate that CBW populations were at the highest levels measured in a decade. The *B.t.* cotton killed greater than 80% of these hatching CBW, but 20% caused injury above the tolerable level. Because the volume of hatching CBW was so great, there were "escapes". As non-*B.t.* corn began to senesce after producing two generations of *Helicoverpa zea* (Boddie) (also known as corn earworm CEW), nearby cotton acreage experienced extremely heavy CBW infestation, especially in areas with high corn acreage. CBW larvae were able to survive feeding on pollen material and then move to bolls lower in the plant canopy where expression of the Cry1Ac protein is lowest. Coupled with the natural tolerance of CBW to the Cry1Ac protein compared to TBW, it is likely that a proportion of the population survived on pollen and grew large enough to escape higher levels of the protein in other tissues. Supplemental insecticide sprays to control CBW were used in some instances, but not all. The results of these investigations are summarized in EPA's 1998 White Paper.[57] EPA is aware of no data or "in-field" evidence of TBW or CBW resistance having developed in the past four years.[58]

While the focus has been on the control of the TBW/CBW complex in the majority of cotton growing areas located from Texas eastward, PBW is the major target insect of *B.t.* cotton in Arizona, California, and New Mexico cotton-growing areas. In addition to Monsanto's required efforts to respond to putative reports of resistance, a multi-agency Rapid Response Team consisting of representatives from the University of Arizona, Arizona Department of Agriculture, the Arizona Cotton Growers Association, the Arizona Cotton Research and Protection Council, and USDA has been organized to promptly investigate growers' claims of failure of *B.t.* cotton to control PBW in Arizona. Putatively resistant populations will be put into culture and tested for susceptibility to *B.t.* toxin. The Arizona Rapid Response Team has not documented any "in-field" resistance events in the past four years.[59]

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57 EPA Publication 739-S-98-001.

58 Sumerford, D.V., D.D. Hardee, L.C. Adams, and W.L. Solomon, Status of Monitoring for Tolerance to Cry1Ac in Populations of *Helicoverpa zea* and *Heliothis virescens*: Three-Year Summary, in Proceeding of the Beltwide Cotton Conferences (1999).

59 Patin, A.L., T.J. Dennehy, M.A. Sims, B.E. Tabashnik, Y-B. Liu, L. Antilla, D. Gouge, T. J. Henneberry, R. Staten, Status of pink bollworm susceptibility to *Bt* in Arizona, in Proceedings

<u>*B.t.* corn (Cry1Ab, Cry1Ac, Cry9C).</u> *B.t.* corn crops express one of three registered *B.t.* endotoxins, Cry1Ab, Cry1Ac, or Cry9C[60] in either field corn (grown primarily for non-human animal consumption), sweet corn or popcorn (the latter two grown primarily for human consumption). In 1995, at the time of the initial registrations of *B.t.* corn, there was no scientific consensus on the details of the IRM plans necessary for prevention of the development of resistance in the two primary target pests, European corn borer (*Ostrinia nubilalis* (Hübner)), ECB) and corn earworm (CEW). At that time, the putative values for adequate refuge size ranged from 20% to 50% of non-*B.t.* corn or other host plants per farm. While the minimum adequate refuge size or structure could not be determined until further research was conducted, it was thought that market penetration of these crops would be sufficiently slow that considerable non-*B.t.* corn would remain to act as natural refuges while the additional research was conducted. Thus, the initial *B.t.* corn registrants instituted voluntary IRM plans.[61]

<u>of the Beltwide Cotton Conferences</u> (1999).

As part of the mandatory terms and conditions of the *B.t.* plant-pesticide registrations, registrants are required to submit monitoring data on the susceptibility of field-collected insect pests to various *B.t.* proteins. No effects outside the normal ranges of susceptibility to the various *B.t.* proteins have been reported for the tobacco budworm, pink bollworm, or European corn borer. In addition, there have been no reports of changes in susceptibility for the Colorado potato beetle. The cotton bollworm (also known as the corn earworm), however, has a natural tolerance to the Cry1Ac toxin. Some degree of increased tolerance (not resistance) to the Cry1Ac toxin produced in *B.t.* cotton in CBW populations from South Alabama, the Mississippi Delta, Georgia, the Florida Panhandle, and South Carolina has been reported based on laboratory bioassays during the three-year period from 1996 to 1998. But, increased tolerance should not be interpreted as resistance. There is no evidence of field failure of *B.t.* cotton due to either TBW or CBW resistance. These results, however, do indicate that factors selecting for CBW resistance may already be increasing in the field and that continued monitoring and further analysis is necessary. The registrant is investigating these reports further and the Agency will continue its close scrutiny regarding the susceptibility of CBW to the Cry1Ac protein. The Agency is prepared to take appropriate regulatory action, if warranted, as further resistance monitoring data become available.

60 Cry9C is registered for non-food and feed uses only.

61 The registrants agreed to various voluntary refuge requirements. For example, Mycogen indicated a commitment to develop a long-term insect resistance management strategy, provide general insect resistance management "guidance," and recommended that not all corn acres be planted in *B.t.* corn. Novartis indicated a commitment to develop a long-term insect resistance management strategy, provided general insect resistance management "guidance," and informed growers that part of a long-term insect resistance management strategy may be "the

The *B.t.* Cry1Ab and Cry1Ac corn registrations include as a term and condition of registration a monitoring plan for ECB and CEW that includes (1) development of baseline susceptibility responses and a discriminating concentration to detect changes in sensitivity, (2) routine surveillance, and (3) remedial action if there is suspected resistance. The purpose of resistance monitoring is to learn whether a field control failure resulted from resistance or other factors that might inhibit expression of the *B.t.* Cry delta endotoxin. The extent and distribution of resistant populations can be mapped and alternative control strategies implemented in areas in which resistance has become prevalent. If monitoring techniques are sensitive enough to discriminate between resistant and susceptible individuals, it should be possible to detect field resistance before significant loss of efficacy and eliminate any resistant individuals using other control tactics.

Under the terms and conditions of the *B.t.* corn registrations, all registrants must require customers to notify them of incidents of unexpected levels of ECB and CEW damage. Registrants are required to investigate these reports and identify the cause of the damage by local field sampling of the plant tissue and suspect insect populations followed by appropriate *in vitro* and *in planta* assays. Any confirmed incidents of resistance are required to be reported to EPA.[62] Based on these investigations, appropriate remedial action is required to mitigate ECB and/or CEW resistance. These remedial actions include: informing customers and extension agents in the affected areas of ECB and/or CEW resistance, increasing monitoring in the affected areas, implementing alternative means to reduce or control ECB or CEW populations in the affected areas, implementing a structured refuge in the affected areas, and cessation of sales in the affected and bordering counties. All registrants have instructed growers to have regular surveillance programs and report any unexpected levels of ECB and CEW damage.[63]

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maintenance of a refuge where susceptible populations of ECB can escape exposure" to the expressed *B.t.* endotoxin. The Novartis 1997 technical bulletin for Cry1Ab hybrids "encourages" growers to: (1) plant *B.t.* hybrids in large blocks, (2) scout for non-target pest and use IPM strategies, (3) maintain a refuge of non-*B.t.* corn, and (4) monitor for unexpected levels of insect damage in *B.t.* corn. In 1998, Novartis recommended that growers follow the IRM guidance for a 20-30% unsprayed structure refuge and a 40% refuge is sprayed with insecticides as outlined in the USDA North Central Regional Publication 602. In 1997, Monsanto (Cry1Ab)) and Dekalb (Cry1Ac) required growers to sign a grower contract that mandated that growers plant either a 5% unsprayed non-*B.t.* corn refuge or a 20% sprayable non-*B.t.* corn refuge.

[62] Such reporting is also required under FIFRA Section 6(a)(2).

[63] Mycogen investigated three customer calls in 1996 related to incidents of unexpected levels of ECB and determined that none of these was related to Cry1Ab resistant ECB. Two of the calls were from growers who forgot where the Event 176 hybrid corn had been planted and one came from a crop consultant who misidentified common stalk borer feeding for ECB.

In February 1998, EPA requested that the FIFRA SAP subpanel on *B.t.* plant-pesticide resistance management review existing IRM strategies for *B.t.* crops. Following the recommendations of this SAP subpanel, EPA began to mandate specific structured refuge options for new *B.t.* corn registrations (those products registered prior to that time were still expected to implement voluntary refuge options). The specific structured refuge requirements were based on the technical recommendations of the February 1998 FIFRA SAP subpanel and NC-205 research committee. In March 1998, EPA required that Novartis mandate (through grower contracts) a 20-30% unsprayed refuge, or, if treated with non-*B.t.* insecticides, a 40% refuge planted within 0.5 miles of *B.t.* corn fields for its Cry1Ab popcorn. In 1999, EPA required that Monsanto mandate (through grower contracts) a 10% unsprayed or 20% sprayed refuge within close proximity of *B.t.* corn fields in the Corn Belt for its Cry1Ab field corn.[64] Dekalb required (through grower contract) a 10% unsprayed or 20% sprayed refuge within close proximity of *B.t.* corn fields for Cry1Ac field corn. In 1998, EPA required that AgrEvo mandate (through grower contract) at least a 20-30% unsprayed refuge or, if treated with non-*B.t.* insecticides, a 40% refuge planted within 1500-2000 feet of *B.t.* corn fields for Cry9C field corn.

In March 1998, EPA approved the registration of Novartis' Cry1Ab (BT11) sweet corn. EPA mandated specific resistance monitoring requirements for this registration for ECB, CEW, and fall armyworm *(Spodoptera frugiperda* (J.E. Smith)), as well as sales reporting requirements. Specific refuge requirements were not mandated for this *B.t.* sweet corn product because sweet corn harvesting occurs before insects mature. Novartis is required through labeling and technical material to have growers destroy any Cry1Ab sweet corn stalks that remain in the fields following harvest in accordance with local production practices. Stalk destruction is intended to reduce the possibility of any insects, including resistant insects, surviving to the next generation.

EPA required that draft IRM plans be submitted by August 1998 for review. At that time, EPA intended that these plans be finalized in 1999, and implemented in 2001. In 1997 and 1998, the USDA NC-205 regional research committee on ecology and management of European corn borer and other stalk-boring Lepidoptera published IRM recommendations.[65] In 1998, NC-205 recommended

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Mycogen investigated two calls of unexpected damage in 1998. Neither of these reports were related to ECB resistance to the Cry1Ab toxin (Event 176).

64 In 1999, the Novartis Cry1Ab field corn registrations were subject to voluntary IRM requirements. The Novartis 1999 grower guide instructed growers to plant a 20% non-*B.t.* corn refuge that may be treated with non-*B.t.* insecticides. Similarly, the Mycogen 1999 grower guide instructed Cry1Ab field corn growers to plant a 20% untreated non-*B.t.* corn refuge or if treated with non-*B.t.* insecticides, a 40% non-*B.t.* corn refuge.

65 Ostlie, K. R., W. D. Hutchinson, and R. L. Hellmich, *B.t.*-Corn & European Corn Borer: Long-Term Success Through Resistance Management, North Central Regional Extension

at least a 20-30% untreated refuge or 40% treated refuge planted within close proximity (<320 acre section of *B.t.* corn).[66] Draft refuge strategies for all Cry1Ab and Cry1Ac field corn and popcorn products were submitted to EPA in August 1998. In April 1999, registrants submitted final refuge strategies for Cry1Ab and Cry1Ac field corn products developed in association with the National Corn Growers Association (NCGA) plan.[67] The industry/NCGA plan focusses on the implementation of a 20% refuge that may be treated if the level of pest pressure meets or exceeds economic thresholds. The plan encourages planting of the non-*B.t.* corn refuge within one-quarter mile of the *B.t.* corn acreage where feasible, and requires planting the refuge within one-half mile of the *B.t.* corn acreage. If treatment of the refuge is expected, the plan requires planting of the refuge within one-quarter mile of the *B.t.* corn plantings. The plan also stated that a 20% untreated refuge or 40% refuge, if treated, should be planted in Northern cotton areas and a 50% refuge that may be treated should be planted in Southern cotton areas. NC-205 reviewed the April 1999 industry/NCGA insect resistance management plan for Cry1A field corn products and concluded that a 20% sprayed refuge may be adequate in most corn growing areas where economic thresholds for ECB are not regularly exceeded. In other words, NC-205 now states that a 20% infrequently sprayed refuge is acceptable.[68] This would apply to most of the Corn Belt east of the High Plains region. NC-205 indicated, however, that further research regarding the efficacy of a 20% sprayed refuge was needed, especially in higher risk areas such as the High Plains region, in which insecticide use has been historically high.[69] NC-205 also

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Publication NCR 602 (1997); NC-205 Supplemental Report, Supplement to: *Bt* corn and European corn borer: long-term success through resistance management (1998).

[66] NC-205 Supplemental Report, Supplement to: *Bt* corn and European corn borer: long-term success through resistance management (1998).

[67] The Industry/NCGA IRM strategies are available at http//www.ncga.com/.

[68] NC-205 letter to Dr. Janet Andersen dated May 24, 1999. See also R. Hellmich's statement and R. Higgins's statements in the Proceedings of the EPA/USDA Workshop on Bt Corn IRM, June 18, 1999, http://www.epa.gov/pesticides/biopesticides. Both documents are in the Special Docket.

[69] Field corn in the United States is rarely sprayed for ECB or CEW. SWCB is treated with insecticides. On average, only approximately 8% of total U.S. field corn acreage is treated for these pests. In those areas considered high risk, insect damage at or above economic thresholds is common and, thus growers use insecticides more often than elsewhere in the Corn Belt.

noted that all *B.t.* corn should be placed within one half mile of the non-*B.t.* corn refuge, but that refuge plantings within one quarter mile would be even better.[70]

B.t. corn presents an additional concern related to pests that are polyphagous, i.e., pests that feed on more than one crop. The corn earworm (CEW)/cotton bollworm (CBW) is an example of a polyphagous pest. CEW/CBW is a pest of both corn and cotton and early generations may live in corn as CEW and subsequent generations in cotton as CBW during one growing season. It is possible that as many as six generations of CEW/CBW can be exposed to the same or related *B.t.* toxins expressed in *B.t.* corn and *B.t.* cotton, significantly increasing the likelihood of the development of resistance. Because CEW/CBW also feeds on other crops (e.g., soybean and tomato), there is also an increased potential for resistant CEW/CBW to move to other host crops that may be treated with *B.t.* foliar sprays, thus rendering them ineffective. EPA originally restricted the sale or distribution of *B.t.* Cry1Ab and Cry1Ac corn products in certain southern counties and states where most cotton is grown. These sales restrictions were necessary to mitigate the development of resistance to *B.t.* toxins in CEW/CBW populations feeding on both corn and cotton. EPA also requested data to develop appropriate refuge options for areas in which corn and cotton are grown.

For the year 2000, all *B.t.* field corn products will have mandatory structured refuge requirements. EPA mandated that all Cry1Ab field corn products will either have a minimum 20% (treatable) non-*B.t.* corn refuge in the Corn Belt or a minimum 50% (treatable) non-*B.t.* corn refuge if *B.t.* corn is grown in southern cotton-growing areas. Larger refuges ($\geq$ 50% non-*B.t.* corn) for *B.t.* corn grown in southern cotton-growing areas are necessary to mitigate resistance development to *B.t.* toxins in CBW/CEW populations feeding on both corn and cotton (both Cry1Ab and Cry1Ac registrations). The refuge must be planted within ½ mile of the *B.t.* corn fields. In regions of the Corn Belt where conventional insecticides have historically been used to control ECB and SWCB, growers wanting the option to treat these pests must plant the refuge within 1/4 mile of their B.t. corn fields. EPA mandated that Cry1Ac (DBT-418) field corn will have a minimum 20% (treatable) non-*B.t.* corn refuge in the Corn Belt. EPA mandated sales restrictions for Cry1Ac (DBT-418) field corn in southern cotton-growing areas to mitigate resistance development to *B.t.* toxins in CBW/CEW populations feeding on both corn and cotton (Cry1Ab and Cry1Ac registrations). Sales restrictions for Cry1Ac (DBT-418) field corn were originally made in 1997 when the product was registered. In addition, all three registrants of non-high expression corn products (Event 176 and DBT-418) agreed to restrict sales of these products in areas which are routinely treated with insecticide sprays, notably the areas which are jointly infested with SWCB and ECB (mainly parts of Oklahoma, Kansas, Texas, and

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[70] NC-205 letter to Dr. Janet Andersen dated May 24, 1999. See also R. Hellmich's statement and R. Higgins's statements in the Proceedings of the EPA/USDA Workshop on Bt Corn IRM, June 18, 1999, http://www.epa.gov/pesticides/biopesticides. Both documents are in the Special Docket.

Colorado). In February 2000, however, DeKalb indicated to EPA that it will no longer sell its DBT-418 (Cry1Ac) corn hybrids in the 2000 growing season.

Specific regional monitoring plans must be expanded to include European corn borer (ECB), southwestern corn borer (SWCB), and corn earworm (CEW) and be submitted to the Agency by March 31, 2000. Registrants must also conduct annual grower surveys to assess compliance with specific IRM requirements. These IRM requirements provide consistency amongst all Cry1A-expressing field corn products.

For the 2000 growing season, EPA has mandated that Aventis instruct growers to plant a minimum structured refuge of at least 20% non-*B.t.* corn for Cry9C field corn. Cry9C field corn is not toxic to CEW; therefore, a 20% non-*B.t.* corn refuge is appropriate throughout all corn-growing areas including *B.t.* corn grown in cotton-growing areas. Insecticide treatments for control of ECB, CEW, and/or SWCB may be applied only if economic thresholds are reached for one or more of these target pests. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents, crop consultants). Instructions to growers will specify that microbial *B.t.* insecticides must not be applied to non-*B.t.* corn refuges. Requirements for refuge deployment will be described in the Grower Guides/Product Use Guides. Growers must plant the refuge within ½ mile of their *B.t.* corn acreage. In regions of the corn belt where conventional insecticides have historically been used to control ECB and SWCB, growers wanting the option to treat these pests must plant the refuge within ¼ mile of their *B.t.* corn. Refuge planting options include: separate fields, blocks within fields (e.g., along the edges or headlands), and strips across the field. When planting the refuge in strips across the field, growers must be instructed to plant multiple non-*B.t.* rows whenever possible. Previous IRM requirements for Cry1Ab popcorn will remain for the 2000 growing season, i.e., substantial non-*B.t.* corn refuge (20-30% unsprayed/40%, if sprayed) must be planted within 0.5 miles of the *B.t.* popcorn fields.

e. <u>Responses to Petitioners' specific arguments</u>

As discussed above, EPA has undertaken extensive efforts to investigate and address the potential development of pest resistance to registered *B.t.* plant-pesticides. Based on available data and information, and consistent with the recommendations of the FIFRA SAP, other federal and state agencies, and entomological experts in academia and elsewhere, EPA has imposed IRM requirements including, structured refuges, monitoring, and remedial action plans. EPA believes that, with these actions, EPA has, to the extent possible based on current scientific understanding, effectively addressed the potential development of pest resistance to registered *B.t.* plant-pesticides. Petitioners, however, have raised specific arguments as to why EPA's efforts to address the potential for development of insect resistance are insufficient. Petitioners' specific arguments are addressed below.

(1) *[T]ransgenic plants exert high selection pressure on pest populations. Insects have the potential to develop resistance to B.t. crops because the plants maintain*

a constant killing dose throughout the growing season. [U]nlike B.t. sprays which are inactivated over a short time, the selection pressure of B.t. plants on susceptible pest populations will be much higher. (Petition at p. 11-12).

EPA agrees with Greenpeace that it is widely recognized that *B.t.* plant-pesticides are effectively more persistent and active than foliar *B.t.* spray products. Season long expression of *B.t.* plant-pesticides in crops will likely increase the selection pressure and therefore increase the risk of *B.t.* resistance development. Therefore, insect resistance management is considered a key to the sustainable use of *B.t.* toxins in transgenic crops.

Using structured refuges for delaying pest resistance to *B. thuringiensis* is the most promising strategy to manage insect resistance.[71] The high dose/structured refuge strategy has been widely endorsed in the scientific community including multiple SAP subpanels, EPA's White Paper, USDA NC-205 (Research and Extension Entomologists of the North Central Regional Research Project (NC-205), Ecology and Management of European Corn Borer and Other Stalk Boring Lepidoptera), International Life Sciences Institute (ILSI), and Hardee *et al.*, and the Union of Concerned Scientists'(UCS) "Now or Never" Report.[72] The Agency has implemented appropriate IRM plans

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71 Liu, Y.B. and Tabashnik, B.E., Experimental evidence that refuges delay insect adaptation to *Bacillus thuringiensis*, 264 Proc. R. Soc. Lond. B. 605 (1997); Alstad, D.N. and Andow, D.A., Managing the evolution of insect resistance to transgenic plants, 268 Science 1894 (1995); Gould, F., Evolutionary biology and genetically engineered crops, 38 Biosci. 26 (1998); Mallet, J. and Porter, P., Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy?, 255 Proc. R. Soc. Lond. B. 65; (1992); McGaughey, W.H. and Whalon, M.E., Managing insect resistance to *Bacillus thuringiensis* toxins, 258 Science 1451 (1992); Tabashnik, B.E., Evolution of resistance to *Bacillus thuringiensis*, 39 Ann. Rev. Entomol. 47 (1994); Tabashnik, B.E., Delaying insect adaptation to transgenic plants: seed mixtures and refugia reconsidered, 255 Proc. R. Soc. Lond. B. 7 (1994).

72 U.S. EPA Scientific Advisory Panel on *Bacillus thuringiensis* (Bt) Plant- Pesticides, February 9-10, 1998 (Docket Number: OPPTS-00231); U.S. Environmental Protection Agency, The Environmental Protection Agency's White paper on *Bt* Plant-Pesticide Resistance Management (EPA Publication 739-S-98-001); Ostlie, K. R., W. D. Hutchinson, and R. L. Hellmich, *Bt*-Corn & European Corn Borer: Long-Term Success Through Resistance Management, North Central Regional Extension Publication NCR 602 (1997); NC-205 Supplemental Report, Supplement to: *Bt* corn & European corn borer: long-term success through resistance management, NCR Publication 602 (1998); International Life Sciences Institute, An evaluation of insect resistance management in Bt field corn: a science-based framework for risk assessment and risk management; Report of an expert panel (1999); Hardee, D.D., J.W. Van Duyn, M.B.Layton, and R.D. Bagwell, *Bt* cotton for management of tobacco budworm and

based on the high dose/structured refuge strategy of resistance management, the recommendations of various scientific expert groups, and continually reevaluates whether these plans are adequate, as new scientific information becomes available.

The high dose expression of the *B.t.* toxin in plants will kill all but rare homozygous recessive (resistant) individuals. In principle, refuges of non-*B.t.* crops enable survival of susceptible individuals, which decreases the intensity of selection and slows evolution of resistance. Under ideal conditions, relatively large numbers of susceptible individuals from refuges survive and mate with few resistant survivors from treated areas. Projections from computer simulations suggest that if resistance is recessive and mating is random, refuges can greatly delay insect adaptation to *B. thuringiensis*.[73] Therefore, a high dose coupled to a structured refuge will effectively dilute resistance in the insect population.

While a high dose is preferable for all target pests, not all registered *B.t.* endotoxins are expressed in transformed plants at high doses.[74] Effective insect resistance management is still possible

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bollworm: Continued effectiveness by managing resistance (Hardee, D.D. and J.W. Van Duyn, eds., in press); Mellon, M. and J. Rissler, Now or never: Serious new plans to save a natural pest control, (M. Mellon and J. Rissler, eds., 1999).

73 Alstad, D.N. and Andow, D.A., Managing the evolution of insect resistance to transgenic plants, 268 Science 1894 (1995); Gould, F., Evolutionary biology and genetically engineered crops, 38 Biosci. 26 (1998); Mallet, J. and Porter, P., Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy?, 255 Proc. R. Soc. Lond. B. 65; (1992); McGaughey, W.H. and Whalon, M.E., Managing insect resistance to *Bacillus thuringiensis* toxins, 258 Science 1451 (1992); Tabashnik, B.E., Evolution of resistance to *Bacillus thuringiensis*, 39 Ann. Rev. Entomol. 47 (1994); Tabashnik, B.E., Delaying insect adaptation to transgenic plants: seed mixtures and refugia reconsidered, 255 Proc. R. Soc. Lond. B. 7-12 (1994).

74 Examples of *B.t.* crops that do not express *B.t.* endotoxin at high levels and, thus, do not provide a high dose to particular target pests are Dekalb's Cry1Ac corn and Mycogen's and Novartis' Event 176 Cry1Ab corn. For the 2000 growing season, Dekalb, Mycogen, and Novartis have agreed to specific mitigation measures to address the Agency's concerns regarding IRM with respect to such non-high dose products. Pending the Agency's comprehensive public review process of all existing *B.t.* registrations in 2000, these measures may be specifically required as terms and conditions of any future registrations of such non-high dose products.

even if the transformed plant doesn't express the *B.t.* endotoxin at a high dose.[75] If the transformed plant doesn't express the *B.t.* endotoxin at a high dose, the IRM plan should include significantly increased refuge size, increased scouting and monitoring, and/or prohibition of sales of the non-high dose product in certain areas.

As discussed below, insect resistance has occurred to *B.t.* foliar spray formulations around the world even though *B.t.* microbial spray formulations have a lower persistence of *B.t.* endotoxins than do *B.t.* plant-pesticides. Moreover, depending on the number of *B.t.* microbial spray applications, frequency, area covered, and life-stage targeted, *B.t.* microbial sprays can impose a high selection pressure on the target insects, a level which in practice may pose an even greater selection pressure on the target pest than the relatively high levels of *B.t.* toxin (i.e., dose) produced in *B.t.* crops.

(2) *Since the initial laboratory and field tests on transgenic plants that express B.t. toxins, it has been reported that several common species of insect pests have evolved resistance to B.t. endotoxins, indicating that biological pesticides can suffer the same fate as synthetic chemicals.* (p. 12)

In the paper that Petitioners cite in support of this argument,[76] the authors state that " in a 1992 laboratory study, eight species were analyzed for resistance to *B.t.* endotoxins." Thus, these studies investigated the potential for development of insect resistance in the laboratory to the chemical *B.t.* endotoxins - not to *B.t.* plant-pesticides expressed in the field. To date, only one of these species, the diamondback moth, has developed field resistance to *B.t.* and this resistance was due to excessive and intensive use of *B.t.* foliar sprays.[77] EPA agrees that there have been many attempts to select insects for resistance to single *B.t.* toxins in the laboratory that have resulted in strains with >100-fold resistance, but there have also been cases of limited or no response to selection in the laboratory.[78] No field resistance to *B.t.* delta endotoxins produced in crops (either potato, corn, or cotton) has been documented. The rate of diamondback moth resistance development cannot be related to the evolution of resistance by other insect species, such as European corn borer, Colorado potato beetle, tobacco

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[75] Gould, F., Sustainability of insecticidal cultivars: integrating pest genetics and ecology, 43 Ann. Rev. Entomol. 701, 719 (1998) ("If we are to use TICs [transgenic insecticidal crops] with moderate expression in a sustainable manner, refuge size must be significantly increased.").

[76] McGaughey and Whalon (1992) (cited by Petitioners at 12).

[77] Tabashnik, B. E, Evolution of Resistance to *Bacillus thuringiensis*, 39 Annu. Rev. Entomol. 47 (1994).

[78] Bauer, L.S., Resistance: a threat to the insecticidal crystal proteins of *Bacillus thuringiensis*, 78 Fla. Entomol. 415 (1995); Tabashnik, B. E, Evolution of Resistance to *Bacillus thuringiensis*, 39 Ann. Rev. Entomol. 47 (1994).

budworm, etc., exposed to *B.t.* plant-pesticides expressed in *B.t.* crops. EPA agrees with the finding that *B.t.* resistance in the laboratory is possible, and in no way disputes or should be perceived to dispute that the possibility of insect resistance to *B.t.* endotoxins expressed in plants is a possibility. The point, however, is that EPA has consistently acted to address resistance issues in accordance with the science. EPA has maintained constant vigilance in staying abreast of the most recent findings and studies. In addition, EPA has undertaken extensive efforts to develop analyses of the available scientific data and information to inform its regulatory positions. EPA has imposed reasonable, scientifically supported IRM requirements as terms and conditions on the relevant registrations that EPA believes effectively addresses the potential development of resistance. EPA will continue to investigate and analyze the current science, and, when necessary, will take regulatory action to address potential resistance problems.

(3) *It was found that in tobacco budworms, a major cotton pest, 1 in 350 individuals carried an allele for resistance to B.t. toxin, a frequency considerably higher than assumed in earlier, theoretical models. The authors predict that with a 4% refuge as mandated by EPA, the B.t. cotton could remain efficacious to tobacco budworm for 10 years, but with other pests such as cotton bollworm and European corn borer, the resistance could develop within 3- 4 years.* (p. 12)

Assuming that the resistance alleles are at least partially recessive and the initial resistance allele frequency is 1.5 X 10^{-3} (1 out of 350 individuals carrying the resistance allele), and a 4% refuge to maintain susceptible moths is followed, then Gould *et al.* conclude that it "should take at least 10 years before *B.t.* resistance becomes a problem in *H. virescens* populations."[79] EPA agrees with Gould's conclusion. As stated above, however, EPA will continue to investigate and analyze the current science and, when necessary, will take regulatory action to mitigate and address resistance problems.

Moreover, EPA does not agree with Petitioners' conclusion that *B.t.* resistance could develop within 3-4 years with other pests such as CBW/CEW and ECB. CBW/CEW and ECB are very different biologically from tobacco budworm. It cannot be assumed that there is or will be a partially recessive *B.t.* resistance gene in these pest species that also occurs at 10^{-3}, the same resistance allele frequency as tobacco budworm (*Heliothis virescens*). Current monitoring data draw from a limited number of acres and cannot be used at this present time to confidently predict resistance gene

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[79] Gould, et al., Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens*, 94 Proc. Nat'l Acad. Sciences USA 3519, 3522. Gould's 1986 model was used to predict the number of years until resistance would occur. Gould, F., Simulation models for predicting durability of insect-resistant germ plasm: a deterministic diploid, two-locus model, 15 Environ. Entomol. 1 (1986).

frequencies. The NC-205 report dated October 1998[80] states that the initial frequency of *B.t.* resistance genes in ECB is probably less than 10^{-3} in parts of Minnesota, Iowa, and Illinois.[81] No resistance allele frequency data are available for cotton bollworm/corn earworm. After four years of commercialization, there has been no evidence that ECB resistance to Cry1Ab or Cry1Ac is occurring in the field, and EPA's approved IRM plans will mitigate the potential for development of resistance.

EPA recognized that there wasn't a high dose of the *B.t.* toxin in either *B.t.* corn or *B.t.* cotton products for CBW/CEW. The Agency imposed reasonable, scientifically-supported IRM requirements as terms and conditions on the relevant registrants that EPA believes effectively addresses the potential development of resistance. In 1999, EPA mandated a 50% sprayable refuge if *B.t.* corn is grown in cotton-growing areas as a means of mitigating the development of cotton bollworm/corn earworm resistance. In 1995, EPA mandated that either a 4% unsprayed refuge or 20% sprayed refuge must be deployed when using *B.t.* cotton. Moreover, there are potential non-cotton hosts that may contribute to the refuge for cotton bollworm and the use of alternative mode of actions, such as pyrethroid oversprays late in the season to kill surviving cotton bollworm, may reduce the survival of potentially resistant cotton bollworm in *B.t.* cotton fields. Despite the moderate dose for cotton bollworm/corn earworm, there is no evidence that resistance to Cry1Ac has occurred in the field after four years of commercial use.[82]

EPA has maintained constant vigilance in staying abreast of the most recent scientific studies and findings. In addition, EPA has undertaken extensive efforts to develop analyses of the available scientific data and information to inform its regulatory positions. EPA will continue to investigate and analyze the current science as it reevaluates the current IRM requirements as part of the comprehensive

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80 NC-205 Supplement, Supplement to : *Bt* Corn & European Corn Borer: Long-Term Success Through Resistance Management, NCR-602 (October 1998). http://ent.agri.umn.edu/ecb/nc205doc.htm.

81 See also Andow, D. A., and W.D. Hutchison, *Bt* corn resistance management, in Now or Never: Serious new plans to save a natural pest control, pp. 19-66 (Union of Concerned Scientists 1998); Andow, D.A., D.N. Alstad, Y. H. Pand, P.A. Bolin, and W.D. Hutchison, Using the F2 screen to find *Bt* resistance genes in European corn borer (Lepidoptera: Crambidae), 91 J. Econ. Entomol. 579-584 (1998); Pierce, C., R. Weinzierl, and K. Steffey, First-year results of a survey for Europan corn borer resistance to *Bacillus thuringiensis*, in: Proceedings of the Illinois Agricultural Pesticides Conference, pp. 67-68 (Cooperative Extension Service, College of Agricultural, Consumer and Environmental Sciences, University of Illinois at Champaign-Urbana January 6-8, 1998).

82 Sumerford, D.V., D.D. Hardee, L.C. Adams, and W.L. Solomon, Status of Monitoring for Tolerance to Cry1Ac in Populations of *Helicoverpa zea* and *Heliothis virescens*: Three-Year Summary, in 1999 Proceeding of the Beltwide Cotton Conferences (1999).

reassessment process, and, if necessary, will make changes to existing IRM requirements and take regulatory action to address potential resistance problems.

(4) *Another recent study demonstrates that the frequency of a multiple-toxin resistance allele in susceptible populations of the diamondback moth is 10x higher that the most widely cited estimate for the upper limit for the frequency of resistance alleles in susceptible populations. The allele can be preserved easily for over 100 generations in the laboratory without exposure to B.t. These findings strongly suggest that the resistance gene carries little, if any, genetic load. Hence, the resistance allele could have far higher frequencies in wild lepidopteran populations than previously thought.* (12)

Petitioners' assertions are based on Tabashnik *et al.*'s recent work regarding the frequency of a multiple toxin resistance allele in a susceptible population of the diamondback moth.[83] EPA disagrees that this study supports a conclusion that the resistance allele could have higher frequencies in wild Lepidoptera than previously thought or that this work necessarily has any relevance to *B.t.* plant-pesticides in field situations. The biology of the diamondback moth is significantly different from that of any of the relevant pests of corn, cotton, or potato. These significant differences, especially increased generation numbers and consequent increased exposure to insecticide, i.e., increased selection pressure, account for the apparent increased ability of the diamondback moth to develop resistance to foliar *B.t.* and many other insecticides.

A close examination of Tabashnik, *et al.* and related information shows that Petitioners' ultimate conclusion, i.e., that *"the resistance allele could have far higher frequencies in wild lepidopteran populations than previously thought,"* must be interpreted with some caution. Tabashnik *et al.* measured the frequency of a *B.t.* resistance allele in LAB-P, which had been developed and reared for >100 generations in the laboratory without exposure to *B.t.* A series of single-pair crosses and bioassays conducted from 1992-1995 showed that a recessive allele conferring resistance to at least four *B.t.* toxins occurred at a frequency of about 10% (0.12) in the LAB-P strain. Tabashnik *et al.* concluded that this frequency is about 10 times higher than the most widely cited estimate for the upper limit for the frequency of resistance alleles in susceptible populations. Further, "extended maintenance of a resistance allele frequency close to 0.10 without exposure to *B.t.* implies that in the absence of *B.t.* heterozygotes have little or no fitness disadvantage relative to susceptibles."[84]

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83 Tabashnik, B. E., Y-B Liu, N. Finson, L. Masson, and D. Heckel, One gene in diamondback moth confers resistance to four Bacillus thuringiensis toxins, 94 Proc. Natl. Acad. Sci. USA 1640 (1997).

84 Id. at 1643.

Petitioners make several subtle, but important mistakes in analyzing the Tabashnik *et al.* report. First, Tabashnik *et al.* studied a single resistant laboratory strain, LAB-P. They did not study "susceptible populations" as stated in the petition.[85] Second, Petitioners conclude that "these findings strongly suggest that the resistance gene carries little, if any, genetic load. Hence, the resistance allele could have far higher frequencies in wild Lepidoptera populations than previously thought."[86] EPA disagrees with Petitioners' conclusion that the resistance allele frequency measured by Tabashnik in this single study involving a susceptible laboratory strain of diamondback moth is sufficient to support a sweeping generalization concerning all other wild lepidopteran populations. In fact, Tabashnik *et al.* conclude that "the frequencies of multiple-toxin resistance genes in other populations of diamondback moth and in other pests remain to be measured."[87] Tabashnik explains that this is an important caveat because "meaningful generalizations about resistance allele frequencies require additional direct empirical estimates."[88] It is not possible to estimate the resistance allele frequency in field populations of insects from a laboratory strain. Thus, EPA believes that it is inappropriate to make generalizations about the resistance allele frequencies in any of the pests that are relevant to *B.t.* crops (e.g., Colorado potato beetle, European corn borer, corn ear worm/cotton bollworm, tobacco budworm, and pink bollworm) based on the frequency of a resistance allele in laboratory colonies of diamondback moth.

Thus, while EPA acknowledges that the frequency of resistance alleles in wild lepidopteran populations could be higher (or lower) than currently assumed, at the present time, based on the currently available data and information, this conclusion is speculative.

(5) *The resistance of diamondback moth to B.t. has also been reported in fields of the United States and Malaysia, and in greenhouses in Japan. All of these cases were found to be related to the extensive application of B.t. to control diamondback moth.* (13)

EPA agrees with Petitioners that diamondback moth resistance to *B.t.* foliar pesticides is a worldwide problem that is related to extensive application (>50 sprays per year for 4 to 8 years) of *B.t.* foliar sprays to control diamondback moth in crucifers especially in Southeast Asia, and that diamondback moth is resistant to many conventional pesticides. A 1989 report lists more than 100

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85 The LAB-P strain was founded with individuals from Pulehu, Maui as described in a 1990 paper by Tabshnik and colleagues. Tabashnik, B. E., N. L. Cushing, N. Finson, and M. W. Johnson, Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: *Plutellidae*), 83 J. Econ. Entomol. 1671 (1990).

86 Petition at 12.

87 Tabashnik, 94 Proc. Natl. Acad. Sci. USA at 1643.

88 Tabashnik, B.E., Insect resistance to *BT* revisited, 15 Nature Biotechnology 1324 (1997).

cases of resistance to 50 different insecticides by diamondback moth worldwide.[89] Petitioners actually understate the geographic distribution of resistance to *B.t.* in diamondback moth. Field resistance to *B.t.* foliar sprays exists in a number of geographically isolated diamondback moth populations worldwide: U.S. (Hawaii, Florida, New York), Asia (China, Japan, Malaysia, Thailand, the Philippines), and Central America (Costa Rica, Guatemala, Honduras, and Nicaragua).[90]

But, while diamondback moth resistance to *B.t.* foliar sprays is now a recognized problem, such DBM resistance does not constitute sufficient basis to assume that the insect pests that are the targets of *B.t.* plant-pesticides, CPB, ECB, CEW, CBW, TBW, and PBW will develop resistance to *B.t.* plant-pesticides when an appropriate high dose/structured refuge IRM strategy is employed. The biology of the diamondback moth is significantly different from that of any of these pests of corn, cotton, or potato. These significant differences, especially increased generation numbers and consequent increased exposure to insecticide, i.e., increased selection pressure, account for the apparent increased ability of the diamondback moth to develop resistance to *B.t.* foliar sprays and many other insecticides. Worldwide diamondback moth resistance to *B.t.* foliar sprays suggests, however, that closer monitoring and resistance management of *B.t.* foliar pesticides should be encouraged. It also confirms that there is a need to examine insect resistance to *B.t.* plant-pesticides. EPA believes that it has taken prudent measures to manage and mitigate the development of insect resistance to *B.t.* plant-pesticides based on all currently available data and information. Moreover, EPA is committed to ongoing vigilance against the development of resistance to *B.t.* plant-pesticides. EPA will continue to closely monitor the resistance literature and to stay abreast of the evolving understanding of the science of resistance. EPA will take regulatory action to require changes in use practices when the scientific understanding indicates that such actions are appropriate.

(6) *The development of resistance by an insect to a Cry protein often leads to cross-resistance, i.e., the insect is resistant to other Cry proteins as well. For example, insects selected for resistance to CryIA(c) showed resistance to CryIA(a), CryIA(b), Cry IB, CryIC, and CryIIA.* (13)

EPA acknowledges that cross-resistance to multiple *B.t.* toxins by a single insect is a possibility. It has not been shown, however, that the development of cross-resistance is a frequent or a likely phenomenon in insect pests exposed in the field to multiple *B.t.* crops producing different *B.t.* toxins. That is, should resistance to *B.t.* corn, B.t. cotton, or *B.t.* potato occur in the field, the same cross-

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89 Georghiou, G. P. and Lagunas-Tejada, The occurrence of resistance to pesticides in arthropods. an index of cases reported through 1989, at 201-205, FAO, Rome (1991).

90 Liu, Y.B., and B. E. Tabashnik, Experimental evidence that refuges delay insect adaptation to *Bacillus thuringiensis*, Proc. Roy. Soc. Lond. Ser.B 264: 605 (1997); Perez, C. P. And A. M. Shelton, Resistance of *Plutella xylostella* (Lepidoptera: *Pyralidae*), to *Bacillus thuringiensis* in South America, 90 J. Econ. Entomol. 669 (1997).

resistance patterns observed in laboratory- or field-selected colonies of diamondback moth are very unlikely to be observed in tobacco budworm, corn earworm/cotton bollworm, pink bollworm, European corn borer, or Colorado potato beetle because these pests are in different families or orders of insects with different biology and genetics.

Studies of laboratory-selected strains of different insect species and isolated field populations of diamondback moth resistant to *B.t.* endotoxins indicate that there is a genetic potential for cross-resistance to develop to single or multiple *B.t.* toxins in a number of major agricultural insect pests. These studies do not indicate, however, that resistance and consequently cross-resistance will occur. Cross-resistance shows a different pattern in different species or even within species and cross-resistance develops not only after treatment with heterogenous conventional *B.t.* foliar preparations, but in experiments using a single isolated *B.t.* toxin as well. Overall, cross-resistance patterns and their underlying physiological mechanism are very complex and unpredictable, even within a closely related group of toxins and susceptible insects.

Contrary to Petitioners' assertion, the relevant literature does not support significant cross-resistance between Cry1A and Cry1B, Cry1C, and Cry2A. Petitioners cite studies involving the tobacco budworm and diamondback moth that showed some broad-spectrum resistance to Cry1Aa Cry1Ab, Cry1B, Cry1C, and Cry2A.[91]

EPA agrees, in principle, with the Petitioners' statements that *"cross-resistance shows a different pattern in different species or even within species"* and *"cross-resistance develops not only after treatment with heterogenous conventional B.t. preparations but in experiments using a single isolated B.t. toxin as well."* The complexity of cross-resistance within a single species or different species is demonstrated by a wealth of experimental evidence. Gould *et al.*[92] selected a tobacco budworm strain (YHD2) for a high level of resistance to Cry1Ac (approximately 2000-fold). The YHD2 laboratory-selected strain was found to be cross-resistant to Cry1Aa, Cry1Ab, and Cry1F and showed limited cross-resistance to Cry1B, Cry1C, and Cry2A. The YHD 2 gene confers resistance to Cry1A toxins (a, b, and c) as well as Cry1F. Genetic experiments revealed that resistance in the YHD2 strain as encoded is partially recessive and is controlled mostly by a single locus or a set of tightly linked loci.[93] These results are in contrast to Gould *et al.*'s 1992 published work

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[91] Petitioners cite McGaughey W.H., Whalon, M.E.(1992), and Tabashnik, B.E. *et al.*(1997) at 13.

[92] Gould, F., A. Anderson, A. Reynolds, L. Bumgarner, and W. Moar, Selection and Genetic Analysis of a *Heliothis virescens* (Lepidoptera: *Noctidae*) Strain with High Levels of Resistance to *Bacillus thuringiensis* Toxins, 88 J. Econ. Entomol. 1545 (1995).

[93] Id.; Heckel, D.G., L.C. Gahan, F. Gould, A. Anderson, Identification of a linkage group with a major effect on resistance to *Bacillus thuringiensis* Cry1Ac endotoxin in tobacco budworm

using his more moderately-resistant laboratory strain of tobacco budworm (<50-fold) which showed some broad-spectrum resistance to Cry1Aa, Cry1Ab, Cry1B, Cry1C, and Cry2A.[94] The resistance levels in this TBW strain were low, and subsequent work showed that resistance was inherited as a nearly additive trait.[95] These results show that cross-resistance shows a different pattern to a closely related group of toxins by TBW. It is thus difficult to predict what cross-resistance patterns are likely to be in the field because evolutionary responses will depend on the initial frequencies of each resistance allele, the dominance of the alleles, and how the toxins are used.

EPA also acknowledges that Petitioners' assertion that *"due to the heterogeneity of the toxin binding sites in the insect's gut and insufficient knowledge about binding mechanisms such cross-resistances cannot be predicted (e.g. by sequence homologies)"* is correct. Investigation of a resistant diamondback moth population isolated in Florida[96] indicated that the larvae were resistant to the *B.t.* HD-1 spore and to Cry1Aa, Cry1Ab, and Cry1Ac endotoxins. The larvae were susceptible to Cry1B, 1C, and 1D. There was no cross-resistance to Cry1E and Cry2A. Similarly, populations of resistant diamondback moth from Hawaii and the Philippines showed high levels of resistance to one or all three of the Cry1A proteins and no cross-resistance to Cry1B or Cry1C, and nonsensitive responses to Cry1E. The mechanism of resistance in the Florida diamondback moth colony seems to be consistently associated with changes in the *B.t.* toxicity pathways and toxin binding of Cry1Aa,

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(Lepidoptera: *Noctuidae*), 90 J. Econ. Entomol. 75-86 (1997).

94 Gould, F., A. Martinez-Ramirez, A. Anderson, J. Ferré, F. Silva, and W. Moar, Broad Spectrum *Bt* Resistance, 89 Proc. Natl. Acad. Sci. USA. 1545 (1992).

95 Id.

96 Tang, J. D., A. M. Shelton, J. Van Rie, S. De Roeck, W. J. Moar, R. T. Roush, and M. Peferoen, Toxicity of *Bacillus thuringiensis* spore and crystal protein to resistant diamondback moth (*Plutella xylostella*), 62 Appl. Environ. Microbiol. 564 (1996); Ferré, J., M.D. Real, J. Van Rie, S. Jansens, M. Peferoen, Resistance to the *Bacillus thuringiensis* in a field population of *Plutella xyostella* is due to a change in a midgut membrane receptor, 88 Proc. Natl. Acad. Sci. USA 5119 (1991); Tabashnik, B. E., N. Finson, F. R. Groeters, W. J. Moar, M. W. Johnson, K. Luo, and M. J. Adang, Reversal of resistance to *Bacillus thuringiensis* in *Plutella xylostella*, 91 Proc. Natl. Acad. Sci. USA 4120 (1994); Tabashnik, B. E., N. Finson, M. W. Johnson, and W. J. Moar, Resistance to toxins from *Bacillus thuringiensis* subsp. *kurstaki* causes minimal cross-resistance to *B. thuringiensis* subsp. *aizawai* in the diamondback moth (Lepidoptera: *Plutellidae*), 59 Appl. And Environ. Microbiol. 1332 (1993).

Cry1Ab, and Cry1Ac.[97] The authors indicate that resistance to the HD-1 spore and the three Cry1A toxins may have involved 2 separate resistance mechanisms or that one mechanism evolved that conferred resistance to both spore and Cry1A because of some shared attribute. In another study involving diamondback moth, Tabashnik *et al.* determined that a single autosomal recessive gene conferred extremely high resistance to four *B.t.* toxins: Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F, in a diamondback moth isolated in Hawaii.[98] Results presented by Ballester *et al.* indicated that *B.t.* resistance in a field population of diamondback moths from the Philippines is specific for Cry1Ab not to the closely related Cry1Aa and Cry1Ac with which Cry1Ab shares amino acid identity of 90% and 86%, respectively.[99] The conclusion is that within one species, one field *B.t.*-resistant strain may not show the same cross-resistance potential as another field *B.t.*-resistant strain.[100]

European corn borer (*Ostrinia nubilalis* (Hübner)) selected for resistance to Cry1Ab and to Cry1Ac indicate that the Cry1Ac-resistant strain and the Cry1Ab-resistant strain of European cornborer had a moderate level of resistance, about 30 to 60X.[101] None of the resistant larvae survived on *B.t.* corn beyond the second instar and, therefore, would not pass on resistance to the next generation. Also, these results indicate that the Cry1Ac-resistant ECB are not cross-resistant to

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97 Tang, J. D., A. M. Shelton, J. Van Rie, S. De Roeck, W. J. Moar, R. T. Roush, and M. Peferoen, Toxicity of *Bacillus thuringiensis* spore and crystal protein to resistant diamondback moth (*Plutella xylostella*), 62 Appl. Environ. Microbiol. 564 (1996).

98 Tabashnik, B.E. et al., One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins, 94 Proc. Natl. Acad. Sci. USA. 1620 (1997).

99 Ballester, V., B. Escriche, J.L. Ménsua, G.W. Riethmacher, and J. Ferré, Lack of cross-resistance to other *Bacillus thuringiensis* crystal proteins in a population of *Plutella xylostella* highly resistant to Cry1Ab, 4 Biocontrol Sci. And Tech. 437 (1994).

100 Tang, J. D., A. M. Shelton, J. Van Rie, S. De Roeck, W. J. Moar, R. T. Roush, and M. Peferoen, Toxicity of *Bacillus thuringiensis* spore and crystal protein to resistant diamondback moth (*Plutella xylostella*), 62 Appl. Environ. Microbiol. 564 (1996). In addition, a laboratory-selected strain of the cabbage looper (*Trichoplusia ni* (Hübner)) also showed resistance to Cry1Ab, but not the closely-related Cry1Aa and Cry1Ac toxins. Estada, U. and J. Ferré, Binding of insecticidal crystal proteins of *Bacillus thuringiensis* to the midgut brush border of the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: *Noctuidae*), and selection for resistance to one of the crystal proteins, 60 Appl. Environ. Microbiol. 3840 (1994).

101 U.S. EPA. The Environmental Protection Agency's White paper on Bt plant-pesticide resistance management. January 14, 1998. [EPA Publication 739-S-98-001]. Washington, DC: US Environmental Protection Agency (1998).

Cry1Ab and that Cry1Ab-resistant ECB are not cross-resistant to Cry1Ac.[102] While Cry1Ac and Cry1Ab are closely related and one might have assumed they would be cross resistant, these binding studies results indicate that, while two toxins may be closely related, there may be different resistance mechanisms not related to toxin binding.

Beet armyworm (*Spodoptera exigua* (Hübner)) selected for resistance to Cry1C in the laboratory showed broad cross-resistance to: trypsinized Cry1Ab, Cry1C, a Cry1E-Cry1C fusion protein (G27), Cry1H, and Cry2A.[103] *In vitro* binding experiments with brush border membrane vesicles showed a two-fold decrease in maximum Cry1C binding, a five-fold difference in binding affinity (K_d), and no difference in the concentration of binding sites for the Cry1C-resistant insects compared with those from susceptible insects. In contrast, a strain of Egyptian cottonworm *(Spodoptera littoralis* (Boisduval)) selected for resistance to Cry1C was about 500-fold resistant and showed partial cross-resistance to Cry1D, Cry1E, and Cry1Ab toxins and to the parental strain, *Bacillus thuringiensis aizawai* 7.29.[104] No cross-resistance occurred to Cry1F.[105]

Based on the available literature examining the receptor binding properties of Cry1A and Cry2A delta endotoxins in corn earworm/cotton bollworm, tobacco budworm, and European corn borer larvae, it appears to be very unlikely that cross-resistance would develop to Cry2A delta endotoxins if resistance develops to Cry1A or Cry9C delta endotoxins in commercially available *B.t.* corn and *B.t.* cotton. Based on the work of English *et al.*, Cry1A and Cry2A proteins exhibit different binding characteristics and likely possess different modes of action.[106] These authors concluded that Cry2A formed a voltage-dependent rather than cation-selective channel in the membrane unlike Cry1A and Cry3A toxins.[107] Therefore, the Cry2A toxin has a different biochemical mode of action than do

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102 Id.

103 Moar, W. J., M. Pusztai-Carey, H. Van Faasen, D. Bosch, R. Frutos, C. Rang. K. Luo, and M.J. Adang, Development of *Bacillus thuringiensis* CryIC resistance by *Spodoptera exigua* (Hübner) (Lepidoptera: *Noctuidae*), 61 Appl. Environ. Microbiol. 2086 (1995).

104 Müller-Cohn, J., J. Chaufaux, C. Buisson, N. Gilois, V. Sanchis, and D. Lereclus, *Spodoptera littoralis* (Lepidoptera: *Noctuidae*) resistance to CryIC and cross-resistance to *Bacillus thuringiensis* crystal toxins, 89 J. Econ. Entomol. 791 (1996).

105 Id.

106 English, L., H.L. Robbins, M.A. Von Tersch, C.A. Kulesza, D. Ave, D. Coyle, C.S. Jany, S.L. Slatin, Mode of action of CryIIA: a *Bacillus thuringiensis* delta-endotoxin, 24 Insect Biochem. Molec. Biol. 1025 (1994).

107 Id.

Cry1A or Cry3A toxins. There is, however, some evidence for broad cross-resistance (low levels of resistance) to Cry1A and Cry2A in laboratory-selected strains of beet armyworm[108] and tobacco budworm.[109] The Agency will look closely at insect resistance management strategies for *B.t.* cotton and *B.t.* corn lines that express both Cry1A and Cry2A delta-endotoxins and take appropriate regulatory steps to mitigate resistance development.

EPA has reviewed data submitted by Aventis showing that in both diamondback moth and European corn borer, Cry9C recognizes a different binding site from the one recognized by Cry1A toxins.[110] Due to these separate binding sites, there should be a low potential for cross-resistance between Cry9C and Cry1A toxins. EPA has requested that the company provide additional data regarding the cross-resistance potential for other target pests such as southwestern corn borer, black cutworm, corn stalk borer, as well as test other toxins.

The overall conclusion from these examples is that cross-resistance patterns and their underlying physiological mechanisms are very complex and somewhat unpredictable, even within a closely related group of toxins and susceptible insects. Laboratory- and field-selected resistance may be due to different factors. Studies have shown that laboratory-selected resistant strains do not necessarily predict the rates and mechanisms of resistance in the field or whether cross-resistance will occur. Moreover, results indicate that damage to transgenic plants by neonates is not a reliable indicator of survival to adulthood on transgenic plants producing a *B.t.* endotoxin. For example, results with Colorado potato beetle show that neonates (young larvae) with greater than 400-fold resistance to *B.t.* toxin 3A did not survive on *B.t.* potato plants that produce Cry3A.[111] Therefore, in some cases, pests may need extremely high levels of resistance to overcome high concentrations of toxin in *B.t.* plants.[112]

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108 Moar, W.J., M. Pusztai-Carey, H. Van Faasen, D. Bosch, R. Frutos, C. Rang, K. Luo, and M.J. Adang, Development of *Bacillus thuringiensis* Cry1C resistance by *Spodoptera exigua* (Hübner) (Lepidoptera: *Noctuidae*), 61 Appl. Environ. Microbiol. 2086 (1995).

109 Gould, F., A. Martinez-Ramirez, A. Anderson, J. Ferré, F. Silva, and W. Moar, Broad spectrum *Bt* resistance, 89 Proc. Natl. Acad. Sci. USA. 1545 (1992).

110 Lambert, B., et al., A *Bacillus thuringiensis* insecticidal crystal protein with a high activity against members of the family *Noctuidae*, 62 Appl. and Environ. Microbiology 80 (1996).

111 J.M. Wierenga, D.L. Norris, M.E. Whalon, Stage-specific mortality of Colorado potato beetle (Coleoptera: *Chrysomelidae*) feeding on transgenic potatoes, 89 J. Econ. Entomol 1047 (1996).

112 Tabashnik, B.E., R.T. Roush, E.D. Earle, A.M. Shelton, Resistance to *Bt* toxins, 287 Science 42 (2000).

Because of the complexity and uncertainty associated with predicting cross-resistance, the Agency has taken unprecedented measures to evaluate the cross-resistance of pest species to the Cry toxins expressed in *B.t.* plants. EPA required that registrants submit data evaluating the cross-resistance potential of various insect pests to *B.t.* toxins prior to registration. The Agency concluded that there is ample evidence in the public literature, as described above, that cross-resistance to Cry1A toxins based on modifications to the binding receptor is likely for multiple pests despite the stated uncertainties with actually predicting whether cross-resistance to multiple Cry1A toxins will occur under field conditions. Therefore, as a term and condition of both the *B.t.* corn and *B.t.* cotton registrations, EPA required the generation of additional data regarding the cross-resistance potential of the target pests in both the *B.t.* corn and *B.t.* cotton registrations. Cross-resistance potential for Colorado potato beetle exposed to toxins other than Cry3A was also examined by the Agency in its review of the IRM plan for CPB. The Agency concluded that cross-resistance for Colorado potato beetle was not a concern because other commercialized forms of Cry proteins in *B.t.* crops (e.g., Cry1Ab, Cry1Ac, Cry9C, Cry1F) are not toxic to beetles. The 1995 SAP subpanel concluded that cross-resistance potential was low for CPB.[113] It is possible, however, for a CPB resistant to Cry3A produced in *B.t.* potatoes to also be resistant to a microbial *B.t.* foliar spray that produces the Cry3A toxin and *vice versa*.

Cry1Ac-expressing field corn planted in the southern U.S. could jeopardize the efficacy of cotton plants expressing the Cry1Ab toxin, or the efficacy of *B.t.* foliar spray used on vegetable and other crops because *Helicoverpa zea* (corn earworm/cotton bollworm) may develop cross-resistance to both Cry1Ab and Cry1Ac produced by *B.t.* corn and *B.t.* cotton. EPA required *B.t.* field corn grown in cotton growing areas to have a 50% non-*B.t.* corn refuge which can be treated with certain insecticides to minimize resistance/cross-resistance concerns. EPA also requires mandatory structured refuges for *B.t.* corn in the Corn Belt (20% treatable non-*B.t.* corn). For *B.t.* cotton EPA requires either a 96/4 unsprayed or 80/20 sprayed refuge. EPA also mandated that Cry1Ac-expressing (DBT-418) field corn be restricted in cotton growing areas. For *B.t.* corn and cotton, EPA mandated specific resistance monitoring and grower education to identify *B.t.* resistance should it occur. EPA also required that registrants implement an immediate remedial action plan should *B.t.* resistance develop. Should resistant or cross-resistant CEW/CBW appear, registrants must immediately implement the mandated remedial action plans. EPA will take appropriate regulatory action to require changes in insect resistance management practices for *B.t.* crops if, and when, the scientific understanding of cross-resistance sufficiently supports such actions.

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113 Scientific Advisory Panel, Subpanel on Plant-Pesticides, March 1, 1995. A set of scientific issues were considered in connection with Monsanto Company's application for registration of a plant-pesticide containing the active ingredient *Bacillus thuringiensis* subsp. *tenebrionis* delta-endotoxins. (Docket Number: OPP-00401). (1995) The SAP stated that the insect resistance management plan is scientifically credible to manage resistance to Colorado potato beetle.

(7) *Two biochemical models are discussed to explain emergence of resistance: A change in B.t. receptors or changes in the biochemical pathways of proteolytic cleavage of the protoxin. So far, studies with resistant insects point to receptor changes.* (p. 14)

Petitioners are correct that there may be multiple biochemical mechanisms to explain resistance, but *B.t.* resistance may also be due to more than the two biochemical models discussed in the Petition. Heckel described many different potential resistance mechanisms other than effects on receptor binding.[114] High levels of *B.t.* resistance in laboratory strains of many insects and field-resistant populations of diamondback moth, have for the most part, been associated with receptor changes or more accurately, reduced binding of the *B.t.* toxin to midgut target sites. Studies of *B.t.*-resistant field populations and laboratory strains of diamondback moth have found that resistance can result from changes in molecules that bind *B.t.* Cry toxins to brush border membranes of midgut cells.[115] *B.t.* resistance in strains associated with altered midgut binding properties is thought to be controlled by one or a few genes.[116] Laboratory selection studies with single toxins have also shown that pests can evolve broad-spectrum resistance to many *B.t.* toxins based upon mechanisms other than initial binding affinity. Review of the literature indicates that cross-resistance is more likely to occur when toxins share a common binding site; however, cross-resistance to unrelated toxins is possible.[117] Studies of diamondback moth and other insect species suggest that factors other than receptor binding can also contribute to *B.t.* resistance. Two separate studies of resistance using tobacco budworm colonies

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114 Heckel, G., The complex genetic basis of resistance to *Bacillus thuringiensis* toxins in insects, 4 Biocontrol. Sci. Technol. 451 (1994).

115 Van Rie, J., S. Jansens, H. Hofte, D. Degheele, and H. Van Mellaert, Receptors on the brush border membrane of the insect midgut as determinants of the specificity of *Bacillus thuringiensis* delta-endotoxins, 56 Appl. Environ. Microbiol. 1378 (1990); Ferré, J., M. D. Read, J. Van Rie, S. Jansens, and M. Peferoen, Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor, 88 Proc. Natl. Acad. Sci. USA. 5119 (1991).

116 McGaughey, W. H. and R. W. Beeman, Resistance to *Bacillus thuringiensis* in colonies of Indianmeal moth and almond moth (Lepidoptera: *Pyralidae*), 81 J. Econ. Entomol. 28 (1988); Tabashnik, B. E., J. M. Schwartz, N. Finson, and M. W. Johnson, Inheritance of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: *Plutellidae*), 85 J. Econ. Entomol. 1046 (1992); Ballester, V., B. Escriche, J. Mensua, G. Riethmacher, and J. Ferré, Lack of cross-resistance to other *Bacillus thuringiensis* crystal proteins in a population of *Plutella xylostella* highly resistant to CryIA(b), 4 Biocontrol Sci. Tech. 437 (1994).

117 Tabashnik, B. E., Evolution of Resistance to Bacillus thuringiensis, 39 Ann. Rev. Entomol. 47 (1994).

selected in the laboratory for *B.t.* resistance found no relationship between resistance to Cry1Ab or Cry1Ac and toxin-receptor binding.[118] Thus, the mechanism of resistance to *B.t.* toxins could not be explained solely on the basis of loss of binding capacity to membrane binding sites. Similarly, studies involving the cabbage looper (*Trichoplusia ni)* showed that Cry1Ab and Cry1Ac share the same receptor, but that a strain of *T. ni* selected for resistance in the laboratory to Cry1Ab showed no cross-resistance to Cry1Aa or Cry1Ac.[119] Resistance mechanisms in these insect strains may involve changes in post-binding events such as channel formation, leakage, and repair rate.[120]

From the standpoint of resistance management, it cannot be assumed nor predicted, that cross-resistance in nature will extend broadly to many Cry proteins or be more narrowly defined or that the mechanisms of *B.t.* resistance observed in laboratory- and field-selected diamondback moth populations will be what is potentially observed in other insect species such as the target of *B.t.* crops (e.g., tobacco budworm, cotton bollworm/corn earworm, pink bollworm, European corn borer, Colorado potato beetle). Because of the complexity and uncertainty associated with predicting cross-resistance, the Agency has taken unprecedented measures to evaluate the cross-resistance of pest species to the Cry toxins expressed in *B.t.* plants as described previously (see *supra*, pages 40-41). Should cross-resistance to *B.t.* crops occur, EPA will require appropriate and immediate remedial action based on the mandated remedial action plans. EPA will take appropriate regulatory action to require changes in insect resistance management practices for *B.t.* crops if, and when, the scientific understanding of cross-resistance supports such action. Cross-resistance issues will be reevaluated as part of the Agency's reassessment of the current insect resistance management requirements for *B.t.* corn and *B.t.* cotton registrations.

(8) *Resistance genes are inherited autosomal, confined to only a few loci and seem to be inherited in a recessive way.* (P.14)

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118 Macintosh, S. C., T. B. Stone, R. S. Jokerst, and R. L. Fuchs, Binding of *Bacillus thuringiensis* proteins to a laboratory-selected line of *Heliothis virescens*, 88 Proc. Natl. Acad. Sci. USA. 8930(1991); Gould, F., A. Martinez-Ramirez, A. Anderson, J. Ferré, F. Silva, and W. Moar, Broad spectrum *B.t.* resistance, 89 Proc. Natl. Acad. Sci. USA. 1545 (1992).

119 Estada, U. and J. Ferré, Binding of insecticidal crystal proteins of *Bacillus thuringiensis* to the midgut brush border of the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: *Noctuidae*), and selection for resistance to one of the crystal proteins, 60 Appl. Environ. Microbiol. 3840 (1994).

120 Id.

EPA agrees with this statement for the cases in which inheritance of resistance was studied using *B.t.* plants.[121] Liu *et al.* found that pink bollworm resistance to *B.t.* cotton was recessive.[122] This finding differs from the non-recessive resistance to *B.t.* toxins in artificial diet seen in a laboratory-selected strain of European corn borer (ECB).[123] Huang *et al.* indicated that the genetic inheritance of ECB resistance to a foliar B.t. spray (Dipel ES) is conferred by incomplete dominance.[124] But, Tabashnik *et al.* have stated "Huang *et al.* provide no evidence that either larvae from their Dipel ES-resistant strain or heterozygous larvae can survive to maturity on Bt maize, which means that no conclusions can be drawn about inheritance of resistance to Bt maize."[125] There is no field evidence that ECB are resistant to *B.t.* toxins produced in corn after four years of commercialization. The critical point about the inheritance of resistance and its implications for resistance management is whether heterozygotes die on transgenic plants,[126] and, to date, all data indicate that they do.

(9) *The assumption that resistance to different strains of B.t. endotoxin requires separate independent mutations is false. A recent study demonstrated that a single autosomal recessive gene conferred extremely high resistance to four B.t. endotoxins (CryIA(a), CryIA(b), CryIA(c), CryIF) in the diamondback moth. Hence, if resistance develops it cannot be overcome by just switching to a different toxin.* (p.14)

EPA disagrees with Petitioners' conclusion that demonstration of multiple endotoxin resistance in the diamondback moth conferred by a single autosomal recessive gene necessarily means that such resistance cannot be overcome by switching to other *B.t.* toxins. The paper cited by Petitioners, Tabashnik *et al.*,[127] does refute the idea that multiple resistance to the Cry1Aa, Cry1Ab, Cry1Ac, and

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121 See discussion in Liu, Y-B., B.E. Tabashnik, T.J. Dennehy, A.L. Patin, A.C.Bartlett, Development time and resistance to Bt crops, 400 Nature 519 (1999).

122 Id. Pink bollworm were collected from the field and selected for resistance to Cry1Ac in the laboratory.

123 Huang, F., L.L. Buschman, R.A. Higgins, W.H. McGaughey, Inheritance of resistance to *Bacillus thuringiensis* toxin (Dipel ES) in the European corn borer, 284 Science 965 (1999).

124 Id.

125 Tabashnik, B. E., R. T. Roush, E. D. Earle, A. M. Shelton, Resistance to *Bt* toxins, 287 Science 42 (2000).

126 Id.

127 Tabashnik, B.E., Y.B. Liu, N. Finson, L. Masson, D.G. Heckel, One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins, 94 Proc. Natl. Acad. Sci. USA

Cry1F endotoxins in the diamondback moth requires mutations at independently segregating loci. It does not follow, however, that resistance cannot be overcome by switching to a different *B.t.* toxin. Several *B.t.* endotoxins are still very effective against the resistant diamondback moth, notably Cry1C and Cry1B. Thus resistance to Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F in diamondback moth has in fact been overcome by switching to *B.t.* subsp. *aizawai* which produces the Cry1C endotoxin to which the diamondback moth remains susceptible.[128] Therefore, the published literature actually indicates that there are a number of possible toxin switches that can be made for the DBM, although these may be limited. Moreover, as has been discussed above, the biology of the diamondback moth is significantly different from that of any of the target pests of *B.t.* corn, *B.t.* cotton, or *B.t.* potato. EPA is not aware of any data or information suggesting that multiple endotoxin resistance in ECB, CBW/CEW, TBW, PBW, or CPB is conferred by a single autosomal recessive gene.

(10) *This work has even more disturbing implications for polyphagous insects feeding on different crops such as H. zea. For example, H. zea developing resistance to CryIA(a) while feeding on transgenic B.t. cotton would also acquire resistance to CryIA(b) endotoxin of transgenic B.t. corn as well, or, vice versa. Such a population would show a high level of resistance to conventional B.t. sprays as well and could have a devastating effect to organic farmers that depend on foliar B.t. preparations. Together with the findings that resistance gene frequencies are much higher than previously estimated the cross-resistance phenomenon demonstrates that resistance could develop far faster than previously imagined and with more severe consequences.* (p. 14)

EPA agrees that movement of polyphagous insects feeding on different crops such as *Helicoverpa zea* (CBW/CEW) is an important issue for insect resistance management in *B.t.* crops. This is a primary reason why EPA has taken unprecedented measures to require IRM plans and reevaluate these IRM plans based on the collection of scientific data that began early on when potential applicants for plant-pesticide registrations first began to apply for experimental use permits and registrations.

1640 (1997).

[128] Tabashnik, B. E., N. Finson, M. W. Johnson, and W. J. Moar, Resistance to toxins from *Bacillus thuringiensis* subsp. *kurstaki* causes minimal cross-resistance to *B. thuringiensis* subsp. *aizawai* in the diamondback moth (Lepidoptera: *Plutellidae*), 59 Appl. and Environ. Micro. 1332 (1993); Tang, J. D., Shelton, A. M., Van Rie, J. De Roeck, S., Moar, W., Roush, R. T., and Peferoen, M., Toxicity of *Bacillus thuringiensis* spore and crystal protein to the resistant diamondback moth (*Plutella xylostella*), 62 Appl. Environ. Microbiol. 563 (1996).

EPA believes, however, that certain of Petitioners' conclusions are erroneous and overbroad. First, Petitioners err with respect to the endotoxin that *H. zea* (CBW/CEW) would be exposed to. The endotoxin expressed in *B.t.* cotton is Cry1Ac, not Cry1Aa. Second, Petitioners have greatly overstated the evidence relating to the potential for development of resistant and cross-resistant populations of CBW/CEW and the potential consequences on foliar *B.t.* spray pesticides. The currently available data and information on the development of resistance to *B.t.* does not support Petitioners' conclusions. The estimations of resistant gene frequencies by both Tabashnik *et al.* (1997) and Gould *et al.* (1997) are not directly applicable to CBW/CEW.[129] Moreover, the development of cross-resistance cannot be easily predicted based on laboratory-selected experiments for many different insects, including tobacco budworm, or based on findings for diamondback moth. Also, as discussed above, there is some question as to whether the resistance gene frequencies are in fact as high as postulated in the study relied upon by Petitioners. Thus, it is incorrect to state that it has been demonstrated that resistance could develop "far faster" than previously believed with more "severe consequences."

Nonetheless, EPA agrees with Petitioners that the potential for resistance and cross-resistance to certain *B.t.* endotoxins does exist for CBW/CEW and that reasonable steps should be taken to assure that the potential for resistance and cross-resistance development is mitigated. The life cycle of CBW/CEW is such that it may complete one or two generations feeding on corn, and subsequent generations on cotton, when it is present. Thus, if a CBW population becomes resistant to a particular *B.t.* endotoxin expressed in corn, it may also become resistant to the same or a related *B.t.* endotoxin produced in *B.t.* cotton. Thus, EPA has taken unprecedented and extensive measures to manage and mitigate the development of insect resistance to *B.t.* endotoxins expressed in *B.t.* corn and cotton.

EPA concluded that to manage resistance effectively in both *B.t.* corn and *B.t.* cotton, and to develop a long-term, effective resistance management regulatory strategy, specific data were required (including target and secondary pest biology and ecology, population dynamics (modeling), refugia, cross-resistance, baseline susceptibility and discriminating dose determination). EPA required specific research data, refuge development and implementation, resistance monitoring, grower education, and sales reporting as terms and conditions of registration. In addition, EPA imposed specific mitigation measures (e.g., mandatory structured refugia, grower education, resistance monitoring, and remedial strategies) and data requirements as terms and conditions of the *B.t.* corn and cotton registrations. For the year 2000, all *B.t.* field corn and popcorn, *B.t.* cotton, *B.t.* potato products have mandatory structured refuge requirements for the 2000 growing season. EPA's current IRM requirements are in

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[129] Tabashnik, B.E., Y.B. Liu, N. Finson, L. Masson, D.G. Heckel, One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins, 94 Proce. Natl. Acad. Sci. USA 1640 (1997); Gould, et al., Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens*, 94 Proc. Natl. Acad. Sci. USA 3519 (1997).

section II.A.1.d. As discussed above, EPA will conduct a comprehensive reevaluation of the necessary *B.t.* IRM strategies in 2000.

(11) *EPA has defined "resistancy" as "progeny of sampled individuals showing a >30% survival and >25% leaf area damaged in a 5-day bioassay using Cry1Ab-positive leaf tissue and an LC50 in a standard CryIA(b) diet bioassay that exceeds the upper limit of the 95% confidence interval of the mean historical LC50 for susceptible populations as established by an ongoing baseline monitoring program." This definition cannot be translated easily in a resistance factor as it depends on the range of variations in resistance or tolerance in a population. This range can be surprisingly substantial, and this is not reflected in current monitoring programs.* (p. 14)

Initially, in registering *B.t.* potatoes, corn and cotton, EPA recommended for the *B.t.* potato registrations, and required as a term and condition of registration for *B.t.* corn and *B.t.* cotton, a specific monitoring plan, which must include the development of target pest baseline susceptibility responses, development of a discriminating or diagnostic dose concentration to detect changes in each target pest's sensitivity, routine surveillance, and remedial action if there was suspected resistance. Resistance monitoring plans are required to enable EPA to determine whether a field control failure resulted from resistance or other factors that might inhibit expression of the *B.t.* endotoxin in the plant. The resistance monitoring plan should include sampling sites, timetable for development, education of growers on sampling for resistance, collecting specimens to evaluate for resistance, and providing specific recommendations on how to eradicate resistant individuals to prevent survival of a resistant population. The discriminating dose is the screening tool used to monitor changes in baseline susceptibilities to the Cry proteins, i.e., to indicate when resistance may have developed. When these registrations were first granted, however, there were insufficient data to establish registration-specific discriminating doses. Therefore, while data was being developed that would enable the discriminating dose concentration to be developed for all of the target pests, EPA imposed a default definition of resistance in the *B.t.* corn registrations. The default definition was "progeny of sampled individuals showing a >30% survival and >25% leaf area damaged in a 5-day bioassay using Cry1A (Cry9C or Cry3A) leaf tissue and an LC50 in a standard Cry1A diet bioassay that exceeds the upper limit of the 95% confidence interval of the mean historical LC50 for susceptible populations as established by an ongoing baseline monitoring program." Sufficient data has been gathered to develop a discriminating dose for CPB, ECB, TBW, and PBW. Work is ongoing by the registrants to establish the discriminating dose for SWCB, CBW/CEW, and fall armyworm.

The discriminating dose concentration is recognized as sensitive enough to accurately detect shifts in susceptibilities. Tabashnik *et al.* indicated that bioassays that use one optimal or nearly optimal dose, a discriminating or diagnostic dose concentration, to distinguish between susceptible and resistant individuals are more efficient for evaluating resistance than are bioassays that use several concentrations.[130] The mean historical LC_{50} concentration is recognized as a "crude" measurement of detecting changes in baseline susceptibility and was clearly meant to be a fall-back measurement until discriminating dose concentration existed for all target pests. Each baseline susceptibility measurement is specific for a given localized insect population (i.e., portion of a state) within a measured range of variability (within a standard deviation determined by statistical analysis of the data). The discriminating dose concentration is determined from the baseline data for all sampled populations. Second, reliance on a "resistance factor" is not the important feature of a monitoring program as implied by Petitioners. Growers typically are concerned about what percentage of insects are resistant, not how resistant are the insects.[131] That is, it doesn't matter whether the insects are 10-fold or 1000-fold resistant, what matters to growers is that the insects are resistant.

(12) *Contrary to conventional B.t. endotoxin preparations, transgenic B.t. plants have properties which make the development of pest resistance much more likely*. (p. 15)

This argument essentially repeats argument (1).

f. Petitioners' arguments regarding the creation of novel, *B.t.*- expressing weedy plant relatives

Petitioners argue that the registered *B.t.* plant-pesticides will create novel, *B.t.* expressing weedy plant relatives. Petitioners base this argument on the following assertions: (1) *an additional environmental problem associated with transgenic B.t. plants is the possibility of gene flow from the transgenic cultivars to wild native plants which may acquire B.t. genes from cross pollination*; (2) *a Danish study showed that genes inserted into a crop plant could move rapidly into wild, weedy relatives*; (3) *field tests with other genetically engineered crops have also demonstrated a high frequency of gene flow to non-genetically modified variants*; (4) *a recent study demonstrated that between 35% and 72% of normal potatoes planted at distances up to 1.1 kilometers from genetically engineered potatoes contained the transgene*; (5) *the transfer of B.t. genes to wild related species could enhance resistance development in pests that also feed on these wild species*; (6) *rapid spread of transgenic B.t. plants to centers of biological diversity could counteract all efforts to preserve the precious gene reservoirs of these regions*.

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130 Tabashnik, B. E., N. Finson, C. F. Chilcutt, N. L. Cushing, and M. W. Johnson, Increasing Efficiency of Bioassays: Evaluating Resistance to *Bacillus thuringiensis* in Diamondback Moth (Lepidoptera: Plutellidae), 86 J. Econ. Entomol. 635 (1993).

131 Roush, R. T. and G. L. Miller, Considerations for design of insecticide resistance management programs, 79 J. Econ. Entomol. 293 (1986); ffrench-Constant, R.H. and R.T. Roush, Resistance detection and documentation: the relative roles of conventional biochemical assays, pp. 4-38, in Pesticide Resistance in Arthropods, R.T. Roush and B.E. Tabashnik, eds. (1990).

In this response, EPA will address the issue of transgene flow to weedy plant relatives by first summarizing the Agency's current understanding regarding the likelihood of this phenomenon; EPA will then address Petitioners' specific arguments concerning transgene flow.

g. EPA's current understanding of the likelihood of *B.t.* transgene flow to weedy plant relatives

The movement of transgenes from the host plant into weeds and other crops has been a significant concern for EPA due to the possibility of novel exposures to the pesticidal substance. This concern has been considered for each of the *B.t.* plant pesticides currently registered and EPA believes that these concerns have been satisfactorily addressed. The Agency has determined that there is no significant risk of gene capture and expression of any *B.t.* endotoxin by wild or weedy relatives of corn, cotton, or potato in the U.S., its possessions or territories. In addition, the USDA/APHIS has made this same determination under its statutory authority under the Plant Pest Act.[132]

Under FIFRA, EPA has reviewed the potential for gene capture and expression of the *B.t.* endotoxins by wild or weedy relatives of corn, cotton and potatoes in the U.S., its possessions or territories. *B.t.* plant-pesticides that have been registered to date have been expressed in agronomic plant species that, for the most part, do not have a reasonable possibility of passing their traits to wild native plants. Most of the wild species in the United States cannot be pollinated by these crops (corn, potato and cotton) due to differences in chromosome number, phenology[133] and habitat. There is a possibility, however, of gene transfer from *B.t.* cotton to wild or feral cotton relatives in Hawaii and Florida. Where feral populations of cotton species similar to cultivated cotton exist in Florida and Hawaii, EPA has prohibited the sale or distribution of *B.t.* cotton in these areas. These containment measures prevent the movement of the registered *B.t.* endotoxin from *B.t.* cotton to wild or feral cotton relatives in Hawaii and Florida.

On December 8, 1999, EPA convened a FIFRA SAP subpanel to discuss current data requirements related to the environmental fate, non-target organism, and product characterization for protein plant-pesticides. The SAP released its report February 4, 2000.[134] The panel commended and generally supported EPA's current approach to the product characterization data, the need for

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132 7 U.S.C. 150aa-150jj. See, e.g., USDA determinations specified in footnotes 135, 145, 148, 167, and 177.

133 The periodicity or timing of events within an organism's life cycle, e.g., flowering time.

134 Scientific Advisory Panel, Subpanel on *Bacillus thuringiensis* (Bt) Plant-Pesticides, December 8, 1999, Characterization and Non-Target Organism Data Requirements for Protein Plant-Pesticides. The report is available to the public on the SAP web site at www.epa.gov/scipoly/sap, and in the Special Docket for this action.

additional field monitoring regarding impacts to non-target insects, and testing methodology for non-target insects. The panel also provided specific comments regarding program improvements, including the need for additional studies. The specific recommendations in the report are measures that can be included in our current program, and are consistent with many of the scientific enhancements the Agency is already implementing.

EPA's evaluation of the possibility of movement of *B.t.* transgenes into weedy relatives of potato, corn, and cotton are presented below:

(1) Potato EPA has reviewed the potential for gene capture and expression of *B.t.* plant pesticides (only Cry3A has been introduced into potato) by wild or weedy relatives of cultivated potato in the United States, its possessions or territories. Based on data submitted by the registrant and a review of the scientific literature, EPA concluded that there is no foreseeable risk of unplanned pesticide production through gene capture and expression of the *Bacillus thuringiensis* subsp. *tenebrionis (B.t.t.)* Colorado potato beetle control protein gene (Cry3A) in wild relatives of the transformed plant, *Solanum tuberosum* L in the U.S. or its possessions or territories. Tuber-bearing *Solanum* species, including *S. tuberosum*, cannot hybridize naturally with the non-tuber bearing *Solanum* species in the U.S. Three species of tuber-bearing (section *Petota*) wild species of *Solanum* occur in the United States: *Solanum fendleri*, *Solanum jamesii,* and *Solanum pinnatisectum*. But, successful gene introgression into these tuber-bearing *Solanum* species is virtually excluded due to constraints of geographical isolation and other biological barriers to natural hybridization.[135] These barriers include incompatible (unequal) endosperm balance numbers (EBN) that lead to endosperm failure and embryo abortion, multiple ploidy levels, and incompatibility mechanisms that do not express reciprocal genes to allow fertilization to proceed. No natural hybrids have been observed between these species and cultivated potatoes in the U.S.

In the U.S., *S. fendleri* and *S. jamesii* are restricted to high elevation habitats in the continental southwest, far removed from the centers of commercial potato production. Their distribution has been described by Hawkes:

a) *S. fendleri* subsp. *fendleri* Asa Gray. Arizona, Colorado, New Mexico and Texas at 1,600 to 2800 meters in dry oak-pink forest, but not under dense shade.

b) *S. fendleri* subsp. *arizonicum* Hawkes. Arizona in pine forest clearings and roadsides from about 2000-2550 meters.

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135 USDA/APHIS Petition 94-257-01 for Determination of Nonregulated Status for Colorado Potato Beetle-Resistant Potato Lines BT6, BT10, BT12, BT16, BT17, BT18, and BT23. Environmental Assessment and Finding of No Significant Impact (March 2, 1995).

c) *S. jamesii* Torr. Arizona, Colorado, New Mexico, Texas, and Utah.[136]

S. pinnatisectum is reported to be found in Arizona, though it is considered primarily a Mexican species.[137] While somatic hybrids (protoplast fusion) can be made and some of these fusions produced plants that can be backcrossed with potato, it cannot naturally cross with *S. tuberosum* because of abortion of hybrid endosperm.[138]

Even if plants of *Solanum tuberosum* (commercial potato) and either of the three native tuber-bearing species were to grow contiguously, cytological differences in ploidy level and/or endosperm balance number between the wild and cultivated species should bar successful hybridization and gene introgression.[139] Controlled crosses between *S. fendleri* and *S. tuberosum*, for example, have been successful only with intermediate bridging crosses and have produced hybrids incapable of further sexual reproduction.[140] This does not present a risk of spread because intermediate bridging crosses do not occur in nature.

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136 Hawkes. J.G., The potato: evolution, biodiversity and genetic resources (Smithsonian Institution Press 1990).

137 USDA, NRCS. The PLANT database (http://plants.USDA.gov/plants). National Plant Data Center, Baton Rouge, LA 70874-4490, USA (1999).

138 R. Thieme, et al., Production of somatic hybrids between S. tuberosum L. and late blight resistant Mexican wild potato species, 97 Euphitica 189 (1997).

139 Johnston, S.A., T.P.M. den Nijs, S.J. Peloquin and R.E. Hanneman, Jr., The significance of genic balance to endosperm development in interspecific crosses, 57 Theoretical and Applied Genetics 5 (1980); Novy, R.G. and R.E. Hanneman, Jr., Hybridization between gp. Tuberosum haploids and 1EBN wild potato species, 68 American Potato Journal 151 (1991).

140 Soest, L.J. W., The crossability of Solanum tuberosum with two wild species, series Longipedicellata, resistant to late blight, in: Potato research of tomorrow: drought tolerance, virus resistance and analytic breeding methods, Proceedings of an international seminar, Wageningen, Netherlands, 30 October 1985, p. 161 (1986). A summary of Soest's work is a follows: the crossability of *S. hjertingii* and *S. fendleri* with several potato cultivars was very difficult. However, some three-way hybrids were obtained when the cv. Olympia as a female parent was fertilized with a pollen mixture of the cross *S. fendleri* X *S. hjertingii*. These three-way hybrids were further backcrossed with cultivated tetraploids, but relatively low seed set was obtained. Reciprocal hybridization between the two allotetraploids on the one hand and four dihaploids and three wild species of the Series Tuberosa on the other, yielded sterile triploids.

All cultivated potatoes in the U.S. belong to the species, *S. tuberosum*. Although it is possible to produce potatoes sexually from true seed,[141] commercial production of *S. tuberosum* in the United States is done asexually through the use of tubers. The production of fruits by the crop, when it occurs, is only incidental to plant growth necessary for tuber maturation. Therefore, even in cases where an organic grower's non-*B.t.* potato fields are in close proximity to *B.t.* potato fields, cross-pollination would not result in the tubers containing the *B.t.* gene. Seed potato production from such tubers would also be *B.t.* gene free.

Many barriers exist for gene transfer from CPB-resistant potatoes to other potato cultivars or free-living relatives. The widely planted cultivar, Russet Burbank, is male sterile. Other cultivars range from Shepody with "almost nil" pollen shed,[142] and Atlantic, which is also almost male sterile,[143] to the self-fertile variety, Superior. Lack of floral nectaries and low or no pollen production in many cultivars restrict insect-mediated, primarily bumblebee cross pollination.[144] Cross pollination drops to very low levels within a few meters of the pollen source.[145]

Berries produced by self- or cross-fertilization within potato fields have been reported to result in volunteer potato weeds in subsequent crops.[146] Factors reducing the probability of this event include: low self and/or cross fertility among many of the potato cultivars being grown in the United States,

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141 Martin, M.W., Field seeding of true potato seed in a breeding program, in: The Production of New Potato Varieties: Technological Advances, p. 261 (edited by G.J. Jellis and D.E. Richardson) (Cambridge University Press, 1987).

142 Young, D.A., T.R. Tarn and H.T. Davis, Shepody: a long, smooth, white-skinned potato of medium maturity with excellent french fry quality, 60(2) American Potato Journal 109 (1983).

143 Memorandum from W. Schneider to P. Hutton, Office of Pesticide Programs (OPP) Preliminary Scientific Position of the September 3, 1993, Monsanto Company Application for a Registration for Insecticidal Proteins Produced by Foreign Genes in Potato (Solanum tuberosum L.) Plants (January 19, 1995) [OPP Docket , OPP-00401].

144 Arndt, G.C., J.L. Rueda, H.M. Kidane-Mariam, and S.J. Peloquin, Pollen fertility in relation to open pollinated true seed production in potatoes, 67 American Potato Journal 499 (1990).

145 USDA/APHIS Petition 94-257-01 for Determination of Nonregulated Status for Colorado Potato Beetle-Resistant Potato Lines BT6, BT10, BT12, BT16, BT17, BT18, and BT23. Environmental Assessment and Finding of No Significant Impact (March 2, 1995).

146 Lawson, H.M. and J.S. Wiseman., Weed control in crop rotations: volunteer crops, Report of the Scottish horticultural Research Institute for 1980, pp. 43 (1981); Lawson, H.M., True potato seeds as arable weeds, 26 Potato Research 237 (1983).

critical environmental conditions necessary for fruit set, even with fertile cultivars,[147] and competitive disadvantage of seed-produced potatoes in tuber-produced fields.

Therefore, CPB-resistant potatoes are unable to outcross to male-fertile potato cultivars, and the chances for successful cross-pollination of CPB-resistant potatoes by male-fertile potato cultivars and subsequent seed production will be minuscule. The potential for the CPB-resistant potatoes to become an aggressive weed in the U.S. is negligible.[148]

(2) Corn or Maize. EPA has reviewed the potential for gene capture and expression of the Cry1Ab, Cry1Ac, and Cry9C endotoxin genes from *B.t*. plant pesticides, as expressed in corn plants, by wild or weedy relatives of maize in the United States, its possessions and territories. Following this review, EPA believes there is no significant risk of gene capture and expression of any of the Cry endotoxins by wild or weedy relatives of maize in the United States because extant populations of sexually compatible species related to *Zea mays* (maize or corn) are not present in the continental United States or its territories and possessions.[149]

Zea mays is a wind-pollinated species, and the presence of spatially separate tassels (male flowers) and silks (female flowers) encourages outcrossing among nearby plants. Maize cultivars and landraces are known to be interfertile to a large degree. Recent studies have indicated that cross-pollination at 100 ft. from the source of genetically modified maize was 1 % and this proportion declined exponentially to 0.1 % at 130 ft and further declined to 0.03 % at the farthest distance measured (160 ft).[150] For production of Foundation Seed, a distance of 660 ft has been required to ensure separation of pollen types. Additionally, the relatively large size of corn pollen as compared to other grass species and the short time span that corn pollen remains viable (i.e., typically less than 60

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147 Burton, W.G., The Potato, (Third edition, John Wiley & Sons, Inc. 1989).

148 See also -- USDA/APHIS Petition 94-257-01 for Determination of Nonregulated Status for Colorado Potato Beetle-Resistant Potato Lines BT6, BT10, BT12, BT16, BT17, BT18, and BT23. Environmental Assessment and Finding of No Significant Impact. (March 2, 1995).

149 U.S. Environmental Protection Agency, Office of Pesticide Programs, Biopesticides and Pollution Prevention Division, Memorandum from Wozniak, C.A. to M. Mendelsohn, *Tripsacum* and *Zea* species present in the United States and its territories: the potential for hybridization with *Zea mays* (February 16, 2000).

150 Jemison, J. and M. Vayda, University of Maine at Orono, Pollen transport from genetically engineered corn to forage corn hybrids: A case study, Abstract presented to the Maine Agricultural Trade Show, January, 2000.

minutes) under natural conditions both preclude long distance transfer for purposes of outcrossing.[151] Under conditions of high temperature[152] and desiccation,[153] corn pollen longevity is measured in minutes. These conditions may even destroy the anthers before any viable pollen is shed.[154] More moderate conditions can extend the field life to hours.[155]

For transformed plants to become weedy escapes as a result of the genetic modification (i.e., expression of *B.t.* endotoxins that protect plants from insect damage), they would need to inherit and express many other unrelated traits that provide selective advantage to a weedy growth habit (e.g., large numbers of easily dispersed seeds, propensity to grow on disturbed ground, vegetative propagation, seed dormancy, etc.). These traits do not exist within the maize complement of genetic characters, a species that has been selected for domestication and cultivation under conditions not normally found in natural settings. The presence of a large cob or ear that does not shatter as the bearer of seeds severely limits the dispersing abilities of maize and it has been theorized that in the absence of human intervention the species as we know it would die out in a few generations due to competition amongst seedlings germinating from the cob.

Transformation of corn to express *B.t.* endotoxin does not alter the ability of maize to outcross with teosintes (*Zea mays* ssp. *mexicana*, *Z. mays* ssp. *parviglumis*, *Z. luxurians*, *Z. perennis*, *Z. diploperennis*) or *Tripsacum* species. Teosintes exist as special plantings (*e.g*., in research plots, botanical gardens, and greenhouses) and some are used to a small extent as forage crops in the western United States. Many native teosintes in Mexico, El Salvador, Guatemala, Nicaragua and Honduras are interfertile with maize to varying degrees and have been known to produce viable seedlings.[156] Despite

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151 Schoper, John, personal communication, Geneticist, Pioneer Hi-Bred International, Johnston, IA, December, 1999.

152 Herrero, M.P. and R.R. Johnson, High temperature stress and pollen viability of maize, 20 Crop Science 796 (1980).

153 Hoekstra, F.A., L.M. Crowe and J.H. Crow, Differential desiccation sensitivity of corn and Pennisetum pollen linked to their sucrose contents, 12 Plant, Cell and Environment 83 (1989).

154 Lonnquist, J.H. and R.W. Jugenheimer, Factors affecting the success of pollination in corn, 35 Journal of the American Society of Agronomists 923 (1943).

155 Jones, M. D. and L.C. Newell, Longevity of pollen and stigmas of grasses: buffalograss, *Buchloe dactyloides (Nutt.) Engelm.*, and corn, *Zea mays L.*, 40(3) Journal of the American Society of Agronomy 195 (1948).

156 Wilkes, H.G., Teosinte: The closest relative of maize, Bussey Inst., Harvard University, Cambridge, MA. (1967).

having coexisted and co-evolved in close proximity to maize in the Americas over thousands of years, however, maize and teosintes maintain distinct genetic constitutions even with this sporadic introgression.[157] Given the cultural and biological relationships of various teosinte species and cultivated maize over the previous millennia, it appears that gene exchange has occurred (based largely upon morphological characters) between these two groups of plants and that no weedy types have successfully evolved as a result. More recent cytogenetic, biochemical and molecular analysis has indicated that the degree of gene exchange is far less than previously thought and evidence for gene introgression into teosinte from maize may be considered as circumstantial at present.[158]

The teosintes retain a reduced cob-like fruit/inflorescence that shatters more than cultivated maize, but still restricts the movement of seeds as compared to more widely dispersed weedy species. Hence, the dispersal of large numbers of seeds, as is typical of weeds, is not characteristic of teosintes or maize. In their native habitat, some teosintes have been observed to be spread by animals feeding on the plants. Teosintes and teosinte-maize hybrids do not survive even mild winters and would not propagate in the U.S. Corn Belt. Additionally, some types have strict day length requirements that preclude flowering within a normal season (i.e., they would be induced to flower in November or December) and, hence, seed production under our temperate climate.[159]

Based on the ability of maize to hybridize with teosintes, the results of previous genetic exchange amongst these species over millennia, and their general growth habits, any introgression of genes into wild teosinte from *Zea mays* is not considered to be a significant agricultural or environmental risk. The growth habits of teosintes are such that the potential for serious weedy propagation and development is not biologically plausible in the United States.

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157 Doebley, J., Molecular evidence for gene flow among Zea species, 40 BioScience 443 (1990); Doebley, J. F., 1984, Maize introgression into teosinte - A reappraisal, 71 Ann. Missouri Bot. Gard. 1100 (1984).

158 Doebley, J., M.M. Goodman, and C.W. Stuber, Patterns of isozyme variation between maize and Mexican annual teosinte, 41 Econ. Bot. 234 (1987); Kato Y., T.A., Review of Introgression between maize and teosinte, in: Gene Flow Among Maize Landraces, Improved Maize Varieties, and Teosinte: Implications for Transgenic Maize, pp.44, Serratos, J.A., Wilcox, M.C., and Castillo-Gonzalez, F. (Eds.), Mexico, D.F., CIMMYT (1997a); Kato Y., T.A., Plenary session: Analysis of workshop reports and discussions. Group I report, in id. at 94; Serratos, J.A., Wilcox, M.C., and Castillo-Gonzalez, F. (Eds.), Mexico, D.F., CIMMYT. (1997b); Smith, J.S.C., M.M. Goodman, and C.W. Stuber, Relationships between maize and teosinte of Mexico and Guatemala: Numerical analysis of allozyme data, 39 Econ. Bot. 12. (1985).

159 Hugh Iltis, Univ. of WI, personal communication to Dr. Chris Wozniak, EPA/BPPD 2000; H. Garrison Wilkes, Univ. of MA, personal communication (2000).

Sixteen species of *Tripsacum* are known worldwide and generally recognized by taxonomists and agrostologists. Most of the 16 different *Tripsacum* species recognized are native to Mexico, Central and South America, but three occur within the U.S. The Manual of Grasses of the United States reports the presence of three species of *Tripsacum* in the continental United States: *T. dactyloides*, *T. floridanum* and *T. lanceolatum*.[160] Of these, *T. dactyloides*, Eastern Gama Grass, is the only species of widespread occurrence and of any agricultural importance. It is commonly grown as a forage grass and has been the subject of some agronomic improvement (i.e., selection and classical breeding). *T. floridanum* is known from southern Florida and *T. lanceolatum* is present in the Mule Mountains of Arizona and possibly southern New Mexico.

For the species occurring in the United States, *T. floridanum* has a diploid chromosome number of 2n = 36 and is native to Southern Florida. *T. dactyloides* includes 2n = 36 forms which are established in the central and western U.S., and 2n = 72 forms which extend along the Eastern seaboard and along the Gulf Coast from Florida to Texas, but which have also been found in IL and KS; these latter forms may represent tetraploids (x = 9 or 18).[161] *T. lanceolatum* (2n = 72) occurs in the Southwestern U.S. Eastern Gama Grass (*T. dactyloides*) differs from corn in many respects, including chromosome number (*T. dactyloides* commonly n = 18; *Zea mays* n = 10). Many species of *Tripsacum* can cross with *Zea*, or at least some accessions of each species can cross, but only with difficulty and the resulting hybrids are primarily male and female sterile.[162]

T. dactyloides, is considered by some to be an ancestor of *Zea mays* or cultivated maize,[163] while others dispute this, based largely on the disparity in chromosome number between the two species, as well as radically different phenotypic appearance. Albeit with some difficulty, hybrids between the two species have been made.[164] In most cases these progeny have been sterile or viable

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160 Hitchcock, A.S. (revisions by Agnes Chase), *Tripsacum L.* Gamagrass, in Manual of the Grasses of the United States, 790-792 (Miscellaneous Publication 200, U.S. Department of Agriculture, 2nd ed. 1971) (ISBN 0-486-22718-9).

161 John Lambert, Univ. of IL; personal communication to Dr. Chris Wozniak, EPA/BPPD (1999).

162 Galinat, W. C., The Origin of Corn, in Corn and Corn Improvement, 1-31, (3rd ed., American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI., 1988); S. Duvick, personal communication to Dr. Chris Wozniak, EPA/BPPD (1999).

163 Mangelsdorf, P.C. The origin and evolution of maize, in Advances in Genetics, 1:161-207 (Ed. M. Demerec, Academic Press, NY., 1947).

164 Mangelsdorf, P.C. and R.G. Reeves, The origin of Indian corn and its relatives, 574 Texas Agricultural Experiment Station Bulletin (monograph) 1-315. (1939); Chet DeWald, USDA-

only by culturing with *in vitro* 'rescue' techniques. Relatively few accessions of *T. dactyloides* will cross with maize and the majority of progeny aren't fertile or viable even in those that do. In controlled crosses, if the female parent is maize, there is a greater likelihood of obtaining viable seed. When these hybrids have been backcrossed to maize in attempts to introgress *Tripsacum* genes for quality enhancement or disease resistance, the *Tripsacum* chromosomes are typically lost in successive generations.[165]

Even though some *Tripsacum* species occur in areas where maize is cultivated, gene introgression from maize under natural conditions is highly unlikely, if not impossible.[166] Hybrids of *Tripsacum* species with *Zea mays* are difficult to obtain outside of the controlled conditions of laboratory and greenhouse. Seed obtained from such crosses are often sterile or progeny have greatly reduced fertility. Approximately 20% of maize-*Tripsacum* hybrids will set seed when backcrossed to maize, and none are able to withstand even the mildest winters. The only known case of a naturally occurring *Zea - Tripsacum* hybrid is a species native to Guatemala known as *Tripsacum andersonii.*[167] It is 100 % male and nearly 99% female sterile and is thought to have arisen from an outcrossing to a teosinte, but the lineage is uncertain.[168] *Zea mays* is not known to harbor properties that indicate it has weedy potential and other than occasional volunteer plants in the previous season's corn field, maize is not considered as a weed in the U.S.[169] The risk of *Tripsacum* / corn hybrids forming in the field is considered minimal. *Tripsacum* species are perennials and seem more closely related to the genus *Manisurus* than either to corn or teosinte.

Since both teosinte and *Tripsacum* are included in botanical gardens in the U.S., the possibility exists (although unlikely) that exchange of genes could occur between corn and its wild relatives. EPA is not aware, however, of any such case being reported in the United States. Gene exchange between cultivated corn and transformed corn would be similar to what naturally occurs at the present time

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ARS, personal communication to Dr. Chris Wozniak, EPA/BPPD (1999).

165 Duvick, Sue, Doctoral Candidate, Department of Plant Genetics, Iowa State University, Ames, Iowa, personal communication to Dr. Chris Wozniak, EPA/BPPD, November, 1999.

166 Beadle, G., The Ancestry of Corn, 242 Sci. Am. 112 (1980).

167 USDA, APHIS, USDA/APHIS Petition 97-265-01 for Determination of Nonregulated Status for Bt Cry9C Insect Resistant and Glufosinate Tolerant Corn Transformation Event CBH- 351: Environmental Assessment. USDA, APHIS, Riverdale, MD (1997).

168 John Doebley, Univ. of WI, personal communication to Dr. Chris Wozniak (2000).

169 Holm, L., Pancho, J. V., Herberger, J. P., and Plucknett, D. L., A Geographical Atlas of World Weeds, p. 391 (John Wiley and Sons, 1979).

within cultivated corn hybrids and landraces. Plant architecture and reproductive capacity of the intercrossed plants will be similar to normal corn, and the chance that a weedy type of corn will result from outcrossing with cultivated corn is extremely remote. Like corn, *Zea mays* ssp. *mexicana* (annual teosinte) and *Zea diploperennis* (diploid perennial teosinte) have 10 pairs of chromosomes, are wind pollinated, and tend to outcross, but are highly variable species that are often genetically compatible and interfertile with corn. *Zea perennis* (perennial teosinte) has 20 pairs of chromosomes and forms less stable hybrids with maize.[170] Corn and compatible species of teosinte are capable of hybridization when in proximity to each other. In Mexico and Guatemala, teosintes exist as weeds around the margins of corn fields. The F1 hybrids have been found to vary in their fertility and vigor. Those that are fertile are capable of backcrossing to corn. Except for special plantings as noted above, however, teosinte is not present in the U.S. or its territories. Its natural distribution is limited to Mexico, Honduras, Nicaragua and Guatemala *Tripsacum*/maize hybrids have not been observed in the field, but have been accomplished in the laboratory using special techniques under highly controlled conditions.

(3) Cotton. EPA has reviewed the potential for gene capture and expression of the Cry1Ac endotoxin in cotton by wild or weedy relatives of cotton in the United States, its possessions or territories. There is a possibility for gene transfer in locations in Hawaii and Florida, where wild or feral cotton relatives exist. Therefore, EPA required stringent sales and distribution restrictions on *B.t.* crops within these states. These containment measures prevent the movement of Cry1Ac from *B.t.* cotton to wild or feral cotton relatives that exist in Hawaii and Florida.

There are four species of cotton, *Gossypium*, in the United States. Two of them, *Gossypium hirsutum* (upland cotton) and *Gossypium barbadense* (sea island cotton, pulpulu haole, Pima), are used commercially and escaped plants can be found growing in the wild in climates where they can survive the winter, e.g., southern Florida and Hawaii. In addition, two native wild species of *Gossypium* occur in the United States: *G. thurberi* Todaro and *G. tomentosum* Nuttall ex Seeman.[171]

G. thurberi Todaro (*Thurberia thespesiodes* Gray) occurs in the mountains of Southern Arizona and northern Mexico at 2,500 to 5,000 feet (rarely at 7000 feet), and is rather common on rocky slopes and sides of canyons in late summer and autumn.[172] Any gene exchange between plants

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170 Edwards, J.W., J.O. Allen, and J.G. Coors, Teosinte cytoplasmic genomes: I. Performance of maize inbreds with teosinte cytoplasms, 36 Crop Sci. 1088 (1996).

171 Brown, H.B. and J.O. Ware, Cotton (3rd ed., McGraw-Hill Book Company, Inc., 1958); Fryxell, P.A., The Natural History of the Cotton Tribe (Texas A & M University Press, 1979); Munro, J.M., Cotton (Second Edition, John Wiley & Sons, 1987).

172 Memorandum from W. Schneider to P. Hutton, Office of Pesticide Programs (OPP) Final Scientific Position of the November 15, 1991, Monsanto Company Application for an

of *Gossypium hirsutum* and *Gossypium thurberi*, if it did occur, would result in triploid (3x=39 chromosomes), sterile plants because *G. hirsutum* is an allotetraploid (4x = 52 chromosomes), and *G. thurberi* is a diploid (2x = 26 chromosomes). Such sterile hybrids have been produced under controlled conditions, but they would not persist in the wild; in addition, fertile allohexaploids (6x = 78 chromosomes) have not been reported in the wild.[173]

The second wild native species, *Gossypium tomentosum*, occurs in Hawaii on the six islands of Kahoolawe, Lanai, Maui, Molokai, Nihau and Oahu.[174] Upland, Hawaiian and sea island cotton are all tetraploids (4x = 52) that can crossbreed.[175] Introgression has been claimed for what one author considered hybrid swarms of *G. barbadense* X *G. tomentosum*.[176] As *G. tomentosum* may bloom at the same time as domestic cotton, there is no guarantee of either geographic or temporal isolation. For these reasons, EPA imposed stringent sales and distribution restrictions on the registration for cotton expressing the Cry1Ac delta endotoxin grown in Hawaii. The Agency required the following labeling statement to mitigate the potential for the Cry1Ac gene to move from cultivated cotton to *G. tomentosum*:

> **"Not for commercial sale or use in Hawaii. Test plots or breeding nurseries established in Hawaii must be surrounded by either 12 border rows of non-cotton if the plot size is less than 10 acres or 24 border rows if the plot is over 10 acres and must not be planted within 1/4 mile of *Gossypium tomentosum.*"**

Experimental Use Permit for an Insecticidal Toxin Produced by a Bacillus thuringiensis Gene in Cotton Plants (March 27, 1992).

173 Stewart, J.M., Gene transfer between contiguous cultivated cotton and between cultivated cotton and wild relatives: report to Monsanto Company (1991) in: Serdy, Information submitted to the United States Environmental Protection Agency, Office of Pesticide Programs, Registration Division, in support of an application for an experimental use permit to ship and use a pesticide for experimental purposes only. EPA DP Barcode #: 171306 (1991).

174 Stephens, S.G., Native Hawaiian cotton (*Gossypium tomentosum Nutt.*) 18 Pacific Science 385 (1964).

175 Beasley, J.O., The origin of American tetraploid *Gossypium* species, 74 Amer. Nat. 285 (1940); Beasley, J.O., The production of polyploids in *Gossypium*, 31 J. Hered. 39 (1940); Beasley, J.O., Meiotic chromosome behavior in species, species hybrids, haploids, and induced polypoids of *Gossypium*, 27 Genetics: 25 (1942).

176 Stephens, S.G., supra, note 174.

The inability of plants or seeds of either of *G. hirsutum* or *G. barbadense* to survive freezing temperatures restricts their persistence as perennials or recurrent annuals to tropical areas. Feral *G. hirsutum* occurs in parts of southern Florida in the Everglades National Park and the Florida Keys.[177] Cotton is not grown commercially in these areas at this time (cultivated cottons are found in the northernmost portions of the state), but the containment provisions of the initial registration must continue for areas in Florida where feral cotton occurs. Wild cotton is a potential concern as it may increase the spread of resistance in Florida (with intensive vegetable production). EPA imposed sale and distribution restrictions on *B.t.* cotton in Florida, restricting its use to those sites North of Tampa (Route 60). The Agency is satisfied that the planting restrictions on *B.t.* cotton (i.e., no *B.t.* cotton south of Tampa) will mitigate concerns for gene transfer to wild cotton:

"**In Florida do not plant south of Tampa, (Florida Route 60).**"

h. <u>Responses to specific arguments of Petitioners</u>

As discussed above, EPA has assessed each of the *B.t.* plant pesticide registrations for likelihood of transgene movement to weedy relatives. EPA believes that in almost all cases, the likelihood of occurrence of such movement is almost non-existent because compatible weedy relatives of *B.t.* crops either do not occur in the United States or are isolated from areas of commercial production. Where compatible weedy relatives do exist in isolated geographic pockets, EPA has imposed stringent sale and distribution restrictions to prevent even the possibility of transgene movement to weedy relatives. EPA believes that, with these actions, EPA has effectively addressed and mitigated the potential movement of transgenes into weedy relatives. The Petition, however, raises specific arguments as to why such movement is of concern. These specific arguments are addressed below.

(1) *An additional environmental problem associated with transgenic B.t. plants is the possibility of gene flow from the transgenic cultivars to wild native plants which may acquire B.t. genes from cross pollination.* (p. 15)

EPA analyzed the possibility of gene flow from transgenic cultivars expressing *B.t.* plant pesticides to wild native plants which acquire the *B.t.* genes through cross pollination as part of its risk assessment of *B.t.* potato, *B.t.* corn, and *B.t.* cotton as summarized above. EPA has concluded that there is no significant risk of gene capture and expression of the *B.t.* toxin(s) by wild or weedy relatives of corn, cotton, or potato in the U.S., its possessions or territories. The creation of "*B.t.*-enhanced weeds" from *B.t.* gene movement from *B.t.* corn or *B.t.* potato is only a remote possibility in the U.S.

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177 See USDA/APHIS Determination on a Petition 94-308-01p of Monsanto Agricultural Company Seeking Nonregulated Status of Lepidopteran-Resistant Cotton Lines 531, 757, 1076. Environmental Assessment and Finding of No Significant Impact. June 22, 1995.

For *B.t.* cotton, where the possibility of gene movement may exist in certain geographically distinct areas, EPA has mitigated the potential for such movement by imposing strict geographic restrictions on the sale and distribution of *B.t.* cotton.

(2) *A Danish study showed that genes inserted into a crop plant could move rapidly into wild, weedy relatives.* (p. 15)

The studies cited by Petitioners to support this assertion indicate the possibility for transgenes to move from oil seed rape *(Brassica napus)* to weedy *Brassica campestris*.[178] EPA recognizes the possibility for genes to move between *B. napus* and weedy *B. campestris*. At the present time, there are no *B.t.* plant-pesticides registered for use in either *B. napus* or *B. campestris*. Prior to registration and use in the U.S., *B.t.* plant-pesticides produced by oil seed rape/canola would have to be reviewed by the Agency. As part of its review, the Agency would examine the possibility of gene flow and it potential impacts, such as the creation of *B.t.*-enhanced weeds, and the potential impact on non-target organisms if the *B.t.* genes were to move to other plants. Moreover, Timmons et al. state "[O]ur work demonstrates the need for a careful, case-by-case approach to the risk assessment of genetically modified organisms."[179] It is EPA's intent to conduct a "careful, case-by-case" review of the environmental risks associated with the possibility of gene movement from crops producing pesticidal substances to wild or weedy relatives, as part of its plant-pesticide registration procedures.

(3) *Field tests with other genetically engineered crops have also demonstrated a high frequency of gene flow to non-genetically modified variants. A recent study demonstrated that between 35% and 72% of the seeds of normal potatoes planted at distances up to 1.1 kilometers from genetically engineered potatoes contained the transgene.* (p. 15)

The study cited by Petitioners suggests that it is possible for two linked marker genes, NTP II and GUS, to move from transgenic potatoes to a conventional potato variety.[180] The circumstances associated in this study were not representative of field practices in the U.S. In the field, potatoes are vegetatively propagated and are not grown to seed; this limits the possibility of gene flow to occur. Tuber-bearing *Solanum* species, including *S. tuberosum*, cannot hybridize naturally with the non-tuber bearing *Solanum* species in the U.S. Three species of tuber-bearing (section *Petota*) wild species of *Solanum* occur in the United States: *Solanum fendleri, Solanum jamesii,* and *Solanum*

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178 Petitioners' reference 38, citing Jorgensen and Andersen, 1994; Mikkelsen et al, 1996; and Timmons et al. 1996.

179 Timmons, et al., 380 Nature 487 (1996).

180 Petitioners' reference 39, citing Skogmyr, I., Gene dispersal from potatoes to conspecifics: a field trial, 88 Theor. Appl. Genet. 770 (1995).

pinnatisectum. However, successful gene introgression into these tuber-bearing *Solanum* species is also virtually excluded due to constraints of geographical isolation and other biological barriers to natural hybridization.[181]

(4) *The transfer of B.t. genes to wild related species could enhance resistance development in pests that also feed on these wild species.* (p. 15).

At present, EPA believes this is a moot point that is not an issue for currently registered *B.t.* crops. As discussed above, gene transfer of currently registered *B.t.* transgenes is not considered a real possibility in the U.S. and its territories for *B.t.* potato and corn. To the extent that there may be a possibility of outcrossing of transgenes from *B.t.* cotton plants to weedy relatives of domestic cotton, EPA has addressed that possibility by restricting *B.t.* cotton in areas where feral *G. hirsutum* occurs in the Florida Keys and where *Gossypium tomentosum* occurs in Hawaii. EPA believes that these sales and distribution restrictions are sufficient to eliminate the possibility of transgene transfer from domestic cotton.

(5) *Rapid spread of transgenic B.t. plants to centers of biological diversity could counteract all efforts to preserve the precious gene reservoirs of these regions.* (p. 17).

Petitioners' argument regarding "centers of biological diversity" (1) presupposes a number of unarticulated assumptions that appear to be unfounded, and (2) appears to imply that EPA should take regulatory action based on factors that are not relevant to its registration decisions for *B.t.* plant pesticides. First, EPA believes that Petitioners' postulated potential threat to centers of diversity if there is "*rapid spread of transgenic B.t. plants to centers of biological diversity*" is hypothetical on three counts: i) Petitioners appear to assume that *B.t.* plants would spread rapidly in any environment. Such rapid spread in all environments cannot simply be assumed. Whether a *B.t.* plant could spread in any particular environment is dependent upon numerous environmental factors, some of which may be site specific and all of which should be appropriately considered. (ii) Petitioners' arguments assume that possession of a transgenic *B.t.* gene will offer an additional selective advantage to the transformed plant to maintain the gene in the population. Whether a *B.t.* gene confers a selective advantage to a plant also depends, in part, on site specific factors and requires site-specific analysis. (iii) Finally, Petitioners' argument appears to assume that *B.t.* transgenic plants will be commercialized in those countries that are the site of centers of diversity and that suitable control measures will not be taken in those countries. Petitioners present no information supporting this assumption. Further, EPA is not aware of any information supporting a conjecture that there may be regulatory failure in such a country.

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181 USDA/APHIS Petition 94-257-01 for Determination of Nonregulated Status for Colorado Potato Beetle-Resistant Potato Lines BT6, BT10, BT12, BT16, BT17, BT18, and BT23. Environmental Assessment and Finding of No Significant Impact. March 2, 1995.

i. Petitioners' arguments regarding the impact on non-target beneficial organisms

Petitioners argue that the widespread use of transgenic plants expressing registered *B.t.* endotoxins may substantially impact soil ecology and negatively impact non-target beneficial organisms. Petitioners base this argument on the following assertions: (1) *studies submitted to EPA by a registrant have revealed potentially negative impact on natural non-target insect populations*; (2) *a study on the impact of B.t. toxin produced by corn on non-target beneficial organisms demonstrated that the Cry1Ab protein caused significant mortality to Collembola and significantly reduced reproduction of the survivors*; (3) *previous safety testing on spray B.t. has found no effect on non-target organisms because the bacterial protoxin is in an inactive state and requires the target organism to activate the toxin*; (4) *according to information submitted by registrants, the truncated B.t. gene in corn produces three more B.t. proteins; it is therefore highly probable that the toxin-like protein of the transgenic corn can also be activated in insects with non-alkaline intestines*; (5) *a recent study suggests that transgenic B.t. plants may create serious impacts on non-target organisms that feed on pests exposed to the toxins*; (6) *decomposition of plant after harvest may result in the accumulation of B.t. toxins at soil concentrations high enough to constitute a hazard to non-target organisms, such as beneficial insects and other animal classes*; (7) *the addition of transgenic B.t. cotton plants to soil frequently caused significant although transient stimulation of culturable, aerobic bacteria and fungal populations*; (8) *purified B.t. toxins, as produced by transgenic plants, continue to be active for a surprisingly long time in some soils and keep their toxic effects.*

In this response, EPA will address the issue of potential impacts to non-target organisms by first summarizing the Agency's current understanding of the current scientific data and information regarding the ecological impacts of plants expressing *B.t.* endotoxins; EPA will then address Petitioners' specific arguments concerning impacts to non-target organisms.

j. EPA's understanding of the current scientific data and information regarding the ecological impacts of plants expressing *B.t.* plant-pesticides

EPA has conducted ecological risk assessments for all *B.t.* endotoxins expressed in potato, corn, and cotton. These risk assessments have demonstrated that *B.t.* endotoxins expressed in transgenic plants do not exhibit recurring or widespread detrimental effects to a substantial number of individual non-target organisms in populations exposed to the levels of endotoxin found in plant tissue. Published field testing results and field test data submitted to EPA also show minimal to undetectable changes to a substantial number of individuals in beneficial insect populations.[182] In some cases the densities of predatory and non-target insects are reported to be higher on *B.t.* crops than on non- *B.t.* crops. These results are discussed below and are summarized in the individual Fact Sheets for each of the registered *B.t.* endotoxins.[183]

EPA assesses the toxicity of a *B.t.* endotoxin to potentially exposed non-target organisms by single species laboratory testing. If toxicity to a particular species is observed, the amount of exposure is quantified and a risk assessment is performed to determine if adverse effects would be expected at the concentrations used under field conditions. Based upon EPA's risk assessment methodology for determining adverse effects to non-target organisms, detrimental effects to an individual species observed only under laboratory conditions does not constitute a sufficient basis to declare such species at risk, unless the test dose is lower than the amount of toxin found in the field. In the event, however, that toxicity is observed at concentration levels less than that found in the field, field studies are required to assess the actual adverse non-target population effects to a substantial number of individuals in a population (in the field, insects are usually exposed to smaller amounts of toxin than the laboratory test dose because in the field there is a greater choice in diet and because other environmental factors play a role in the field setting, e.g., drought, rain, wind, cold, heat, high humidity - all of which result in insect population and diversity variations; the time of feeding - night, day, morning; choice not to feed on sick or dying prey; feeding on top, middle, bottom of plant or leaves; time spent in/on soil, etc.).

The test non-target organisms are chosen as indicators of potential environmental effects and are similar to those examined for microbial or biochemical pesticides. The choice of appropriate indicator organisms for testing is based on the potential exposure as deduced from data on endotoxin expression in the plant. For *B.t.* plants, EPA has examined the toxicity of the endotoxins to birds, fish, honeybees and certain other beneficial insects. EPA required data on *Collembola* (springtail) and earthworm species where crop residue exposure is a possibility to ascertain effects on beneficial soil invertebrates. In the honeybee study, effects studies on brood as well as adults were required when

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182 Fitt, G.P., Martes, C.L. and Llewellyn, D.L., Field evaluation and potential ecological impact of transgenic cottons in Australia, 4 Biocontrol Sci.Tech. 535 (1994); Orr, D.B., and D.A. Landis, Oviposition of European Corn Borer (Lepidoptera: *Pyralidae*) and Impact of natural Enemy Populations in Transgenic Versus Isogenic corn, 90(4) J. Econ. Entomol. 905 (1997); Pilcher, C.D., M.E. Rice, J.J. Obrycki and L.C. Lewis, Field and Laboratory Evaluations of Transgenic *Bacillus thuringiensis* Corn on Secondary lepidopteran pests (Lepidoptera: *Noctuidae*), 90(2) J. Econ. Entomol. 669-678 (1997).

183 These Fact Sheets have been placed in the Special Docket for this Petition Response.

exposure to the *B.t.* endotoxin in pollen was expected. Evaluations of risk to other non-targets which may be affected by the *B.t.* pollen, such as the Monarch Butterfly, are in progress.[184]

Under normal circumstances, *B.t.* crops require substantially fewer applications of chemical pesticides. This should result in fewer adverse impacts to non-target organisms because application of nonspecific conventional chemical pesticides is known to have an adverse effect on populations of non-target beneficial organisms found living in the complex environment of an agricultural field. Many of these beneficial organisms are important integrated pest management controls (IPM) for secondary pests such as aphids and leafhoppers. The overall result of cultivation of plants expressing *B.t.* endotoxin is that the number of chemical insecticide applications for non-target pest control is reduced (although not eliminated) for crops with multiple pest problems.

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184 A study conducted by Losey, et al., suggested that pollen from genetically modified *B.t.* corn may pose risks to monarch butterfly larvae and other butterfly species that feed on milkweed or other plants. John E. Losey, Linda S. Rayor, Maureen E. Carter, Transgenic pollen harms Monarch larvae, 399 Nature 214 (20 May 1999). Prior to registration of the first *B.t.* plant-pesticides in 1995, EPA evaluated studies of potential effects on a wide variety of non-target organisms that might be exposed to the *B.t.* toxin, e.g., birds, fish, honeybees, ladybugs, lacewings, and earthworms. EPA concluded that these species were not harmed. While EPA was aware of potential adverse effects on some species of Lepidoptera, the Agency did not believe that *B.t.* crops would threaten the long-term survival of a substantial number of individuals in the populations of these species. At that time, EPA also concluded that threatened or endangered species of butterflies and moths would not be at risk from *B.t.* corn crops because they would not be exposed to the *B.t.* toxin. Since the publication of the Nature article, EPA has taken a number of steps to more fully assess and understand the possible risks to monarch butterflies and other butterflies from *B.t.* corn pollen. To help identify actual risks to monarch butterflies, EPA has issued a monarch butterfly adverse effects data call-in from the registrants of *B.t.* corn products under its FIFRA Section 3(c)(2)(B) authority. On December 9, 1999, the Agency presented possible new data requirements, including the monarch question, to a FIFRA Scientific Advisory Panel for their recommendations. In addition, EPA is consulting with monarch butterfly experts and USDA to better understand the effect of *B.t.* corn pollen on monarch butterflies. If unreasonable risks are identified, EPA will take appropriate precautionary steps to reduce the risk to monarch butterflies. Until more definitive data and information are available about the potential risks that *B.t.* corn pollen may pose to monarch butterflies and other lepidopterans, EPA is requesting that registrants instruct their customers who are planting refugia beside *B.t.* corn, to the place the refugia upwind and/or between the *B.t.* corn and sensitive habitats for non-target lepidopterans, e.g., roadsides and ditchbanks, as a precautionary measure.

At present, EPA is aware of no identified significant adverse effects of *B.t.* endotoxins to a significant number of non-target beneficial organisms in a population in the field, whether they are pest parasites, pest predators, or pollinators. Published field testing results and field test data submitted to EPA show minimal to undetectable changes in the beneficial insect populations.[185] A study of the feeding of lady beetles on aphids on *B.t.* potatoes did not show any detrimental effects (pre-publication data, R. Roush, Australia).[186] Densities of predatory and non-target insects are generally higher on *B.t.* crops than non-*B.t.* crops solely because the crops are not subjected to the same number of applications of spraying with nonspecific pesticides.

Thus, EPA currently believes that transgenic plants expressing *B.t.* plant-pesticides reduce adverse effects on non-target organisms because the organisms likely to receive a dose of the endotoxin are those feeding on the crop, which, typically, are the phytophagous insects sought to be controlled. While EPA acknowledges the potential for some non-target organisms to be affected as a result of feeding on larvae that have fed on the *B.t.* plants, EPA is not aware of any data or information indicating that any unreasonable adverse effects have occurred. Moreover, *B.t.* endotoxins are characterized by specific toxicity. Thus, the range of insects adversely affected by a particularly *B.t.* toxin is limited. As a consequence, the likelihood that a non-target organism will be adversely affected by exposure to a *B.t.* endotoxin is limited.

Comments provided by members of the 1995 SAP encouraged the use of *B.t.*-potatoes, because of the preservation of beneficial insects. These comments explained that use of *B.t.* crops is a sound IPM strategy especially in areas where CPB infestation is high. In addition, there is a high survival of beneficial insects in association with the use of the potato variety expressing *B.t.* endotoxin. Two members of the panel commented that survival of beneficial insects will likely lead to a reduction in the use of chemical insecticides to control aphids and leafhoppers. A number of entomologists

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185 Fitt, G.P., Martes, C.L. and Llewellyn, D.L., Field evaluation and potential ecological impact of transgenic cottons in Australia, 4 Biocontrol Sci.Tech. 535 (1994); Orr, D.B., and D.A. Landis, Oviposition of European Corn Borer (Lepidoptera: *Pyralidae*) and Impact of natural Enemy Populations in Transgenic Versus Isogenic corn, 90(4) J. Econ. Entomol. 905 (1997); Pilcher, C.D., M.E. Rice, J.J. Obrycki and L.C. Lewis, Field and Laboratory Evaluations of Transgenic *Bacillus thuringiensis* Corn on Secondary lepidopteran pests (Lepidoptera: *Noctuidae*), 90(2) J. Econ. Entomol. 669-678 (1997).

186 Dogan, E.B., R. E. Berry, G.L. Reed, and P.A. Rossignol, Biological parameters of convergent lady beetle (Coleoptera: *Coccinellidae*) feeding on aphids (Homoptera; *aphidae*) on transgenic potato, 89 J. Econ. Entomol. 1105 (1996).

commented on how this potato variety expressing *B.t.* endotoxin can be used with a number of IPM strategies including crop rotation.[187]

When it initially reviewed the applications for the products that were registered in 1995, EPA considered requiring studies evaluating effects upon the representative soil organisms Collembola (springtail) and earthworms. EPA was concerned (1) that such soil organisms may be subject to long-term exposure as a result of soil incorporation of crop residues or when crop residues are left on the soil surface and (2) that adverse effects on such soil organisms could result in an accumulation of plant detritus in cotton fields. Upon reconsideration, however, available information on current routine agronomic practices indicated that the long term soil use of chemical insecticides, such as aldicarb, terbufos, phorate and carbofuran, which have long term adverse effects on soil organisms, has not resulted in the accumulation of significant amounts of plant detritus in soils. Thus, *B.t.* crops, which are expected to have less impact on these species than chemical pesticides, should not result in any increased build up of plant detritus. Supporting this conclusion are data which indicate that endotoxin production ceases at plant senescence, which allows time for protein degradation prior to harvest. Additionally, the environmental fate data indicate that only <1 to 90 grams of *B.t.* protein per acre would enter the soil as a result of post harvest incorporation of *B.t.* plants, and since such proteins are known to degrade rapidly in field soils, the potential for significant soil buildup and effects to non-target soil organisms is not anticipated. This has been confirmed by in-house data,[188] published single species studies,[189] and the field studies cited earlier.[190]

On December 8, 1999, EPA convened a FIFRA SAP subpanel to discuss current data requirements related to the environmental fate, non-target organism, and product characterization for

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187 Subpanel of the FIFRA Science Advisory Panel's (SAP) held March 1, 1995 (final report dated March 16, 1995).

188 These data and EPA's evaluations are discussed in the *B.t.* corn Fact Sheets.

189 Yu, L., R.E. Berry, and B.A. Croft, Effects of *Bacillus thuringiensis* Toxins in Transgenic Cotton and Potato on *Folsomia candida* (Collembola: *Isotomidae*) and *Oppia nitens* (Acari: *Orbatidae*), 90(1) Environ. Entomol. 113 (1997).

190 Fitt, G.P., Martes, C.L. and Llewellyn, D.L., Field evaluation and potential ecological impact of transgenic cottons in Australia, 4 Biocontrol Sci.Tech. 535 (1994); Orr, D.B., and D.A. Landis, Oviposition of European Corn Borer (Lepidoptera: *Pyralidae*) and Impact of natural Enemy Populations in Transgenic Versus Isogenic corn, 90(4) J. Econ. Entomol. 905 (1997); Pilcher, C.D., M.E. Rice, J.J. Obrycki and L.C. Lewis, Field and Laboratory Evaluations of Transgenic *Bacillus thuringiensis* Corn on Secondary lepidopteran pests (Lepidoptera: *Noctuidae*), 90(2) J. Econ. Entomol. 669-678 (1997).

protein plant-pesticides. The Panel's report is available both from the SAP website and the docket for this action. The report is briefly summarized in Appendix B.

k. Responses to Petitioners' specific arguments

As discussed above, EPA does not believe that there are any valid data demonstrating specific adverse impacts of plants expressing *B.t.* endotoxins on beneficial non-target organisms. To the contrary, EPA believes that available scientific data and information indicates that cultivation of *B.t.* crops has a positive ecological effect, when compared to the most likely alternatives. The Petition, however, raises several specific arguments supporting the claim that *B.t.* crops adversely impact beneficial non-target organisms. These specific arguments are addressed below.

(1) *The widespread use of transgenic B.t. plant-pesticides may also substantially impact soil ecology. Although studies have revealed potentially negative impacts on natural non-target insect populations, no further investigations have been demanded by EPA.* (17)

When registrations for *B.t.* endotoxins expressed in transgenic plants were initially being considered, EPA initiated and funded research on the fate and effects on soil of the Cry endotoxins and endotoxin expressing plants.[191] The findings of these studies were considered by EPA in the risk

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[191] Crecchio, C. and G. Stotzky, Insecticidal Activity and biodegredation of the Toxin from *Bacillus thuringiensis* subspecies *kurstaki* bound to humic acids in soil, 30(4) Soil Biol. Biochem. 463-470 (1998); Donegan, K.K., C.J. Palm, V.J. Fieland, L.A. Porteous, L.M. Ganio, D.L. Shaller, L.Q. Bucao, R.J. Seidler, Changes in levels, species and DNA fingerprints of soil microorganisms associated with cotton expressing the *Bacillus thuringiensis* var. *kurstaki* endotoxin, 2 Applied Soil Ecology 111–124 (1995); Donegan, K.K., R.J. Seidler, V.J. Fieland, D.L. Shaller, C.J. Palm, L.M. Ganio, D.M. Cardwell and Y. Steinberger, Decomposition of genetically engineered tobacco under field conditions: persistence of the proteinase inhibitor I product and effects on soil microbial respiration and protozoa, nematode and microarthropod populations, 34 Journal of Applied Ecology 767-777 (1997); Koskella, J. and G. Stotzky, Microbial Utilization of Free and Clay-Bound Insecticidal Toxins from *Bacillus thuringiensis* and their Retention of Insecticidal Activity after Incubation with Microbes, 63(9) Appl. Environ. Microbiol. 3561-3568 (1997); Palm, C.J., K. Donegan, D. Harris and R.J. Seidler, Quantification in soil of *Bacillus thuringiensis* var. *kurstaki* delta-endotoxin from transgenic plants, 3 Molecular Ecology 145–151 (1994); Palm, C.J., D.L. Shaller, K.K. Donegan, and R.J. Seidler, Persistence in soil of transgenic plant produced *Bacillus thuringiensis* var. *kurstaki* delta-endotoxin, 42 Can. J. Microbiol. 1258-1262 (1996); Pilcher, C.D., M.E. Rice, J.J. Obrycki and L.C. Lewis, Field and Laboratory Evaluations of Transgenic *Bacillus thuringiensis* Corn on Secondary lepidopteran pests (Lepidoptera:

assessments associated with the various *B.t.* plant-pesticide registrations. These studies were primarily on soils, or their components, in a laboratory setting. To date there are no reports of any detrimental effects on the soil ecosystems from the use of *B.t.* crops. EPA continues to review adverse effect reports and studies for the purpose of reevaluating the Agency's Ecological Risk Assessment of the *B.t.* crop registrations.

Incorporating the results of these ecological fate and effect studies, EPA established criteria for ecological effects testing of pesticidal crops. Typically, EPA makes a risk assessment of potential recurring or widespread harm to non-target organisms, including plants, based on the use of a pesticide under actual field conditions. FIFRA requires EPA to determine if the benefits of registering a pesticide outweigh the risks associated with use of that pesticide. All pesticides, by definition and intent, have a detrimental effect on some form of life. This will especially be true in laboratory tests at high dosing levels. Therefore, EPA requires registrants to develop non-target organism hazard and environmental fate and expression data. These data support assessments of the exposure and potential risk to wildlife and non-target animal populations and the fate of pesticides in the environment. In the case of *B.t.* plants, the primary concern is exposure of a non-target organism to the expressed *B.t.* endotoxin. If exposure occurs, the toxicity of the endotoxin to the exposed non-target organism must be evaluated. If it is determined that the expressed endotoxin is toxic to the non-target organisms, the amount of exposure is quantified to determine if adverse effects could occur at the concentrations that occur under field conditions. A finding of detrimental effects to single species observed under laboratory conditions, without more, is not sufficient to make a risk assessment. EPA's reviews of the required non-target organism effect data have concluded that the registered plant-pesticides will not have an unreasonable adverse effect on the environment.

Noctuidae), 90(2) J. Econ. Entomol. 669-678 (1997); Saxena, D., S. Forest and G. Stotzky, Insecticidal Toxin in Root exudates from *Bt* corn, 402 Nature 480 (1999); Tapp, H. and G. Stotzky, Insecticidal Activity of the Toxins from *Bacillus thuringiensis* subspecies *kurstaki* and *tenebrionis* Adsorbed an Bound on Pure and Soil Clays, 61(5) Appl. Environ. Microbiol. 1786-1790 (1995); Tapp, H. and G. Stotzky, Dot Blot Enzyme linked Immunosorbent Assay for Monitoring the Fate of Insecticidal Toxins from *Bacillus thuringiensis* in soil, 61(2) Appl. Environ. Microbiol. 602-609 (1995); Tapp, H. and G. Stotzky, Persistence of the Insecticidal Toxin from *Bacillus thuringiensis* subspecies *kurstaki* in soil, 30(4) Soil Biol. Biochem. 471-476 (1998); Venkateswerlu, G., and G. Stotzky, Binding of Protoxin and Toxin Proteins of *Bacillus thuringiensis* subsp. *kurstaki* on Clay Minerals, 25 Current Microbiology 225-223 (1992); Widmer, F., R.J. Seidler and L.S. Watrud, Sensitive detection of transgenic plant marker gene persistence in soil microcosms, 5 Molecular Ecology 603-613 (1996); Widmer, F., R.J. Seidler, K.K. Donegan and G.L. Reed, Quantification of transgenic plant marker gene persistence in the field, 6 Molecular Ecology 1-7 (1997).

Moreover, many of the published reports cited by Petitioners discuss potential hazards that may be *anticipated* on the basis of laboratory data. EPA, however, is not aware of any field data that support any of the conjectured scenarios of ecological risk to beneficial insects or soil. In addition, there is no real evidence that destruction of non-target soil invertebrates from the use of chemical pesticides has had any practical deleterious effects on agricultural soils. It is reasonable to conjecture that there would be an accumulation of organic detritus if soil organisms were significantly impacted. In the 1950's and 1960's, the usage of chlorinated hydrocarbon soil insecticides was widespread, and almost certainly had serious long term deleterious effects on non-target soil invertebrates. Some of these materials had half-lives of 10 or more years. Yet, to EPA's knowledge, there were no reports of reduction in breakdown of plant detritus incorporated into the soil during that period.

(2) *A study on the impact of B.t. toxin produced by transgenic corn on non-target beneficial organisms demonstrated that the CryIA(b) protein caused significant mortality to Collembola and significantly reduced reproduction of the survivors.* (17)

Foliar *B.t.* sprays are not known to cause significant non-target insect population effects, therefore, it may be reasonable to conjecture that *B.t.* endotoxins expressed by transgenic plants also would not cause significant non-target insect population effects. Nonetheless, EPA specifically investigated the potential adverse effects on non-target organisms that might result from *B.t.* endotoxins expressed in plants. EPA has substantial data on non-target organism effects, including effects on mammals, birds, aquatic species, ten insect species from five families, the earthworm and *Collembola* (springtails). These organisms were tested for their susceptibility to the Cry1A, Cry3A, and Cry9C endotoxins expressed in plants. Significant adverse effects were observed only in the lepidopteran pest species. The earthworm and springtails were tested primarily to address possible effects on beneficial soil invertebrates and to address concerns raised by the results of Seidler and Stotzky.[192] Purified *B.t.* endotoxins were not shown to be toxic to parasitic hymenoptera, green lacewing larvae, honeybee larvae, honeybee adults, adult ladybird beetles, springtails, and earthworms when fed up to 10,000 times the level of the endotoxin found in pollen and nectar.[193] These data show that pure Cry endotoxin from plants does not exhibit detectable adverse effects to the major agricultural ecosystem (including the soil ecosystem) beneficial species. A single study conducted by a registrant and cited by Petitioners shows that springtails suffered toxic effects when exposed to *B.t.* endotoxin at rates 191 times higher than would occur under actual field conditions. Because this study had several inconsistencies, and the

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192 Id.

193 These data are discussed in the Fact Sheet for Cry9Cfield corn. The Fact Sheet is in the Special Docket for this action.

fact that *B.t.* endotoxin has not been demonstrated to be toxic to springtails in any other studies, EPA believes this study to be an anomaly.[194]

EPA is unaware of any data or information indicating significant adverse effects of *B.t.* endotoxin expressed in plants to non-target beneficial invertebrates, whether they are earthworms, springtails, parasites, predators, or pollinators. Published field testing results and field test data submitted to EPA show minimal to undetectable changes to a substantial number of beneficial insects in a population. EPA is, however, continuing to research and review the pertinent scientific literature as the science evolves. EPA will take regulatory action to require changes in use practices when the scientific understanding indicates that such actions are appropriate.

(3) *Previous safety testing on spray B.t. has found no effect on non-target organisms because the bacterial protoxin is in an inactive state and requires the target organism to activate the toxin. In contrast, genetically manipulated corn plants, which contain an artificial, truncated B.t. gene, produce a toxin-like protein which is already about half the size of the bacterial protein. It only needs a minor step to turn it into an active toxin. It seems that there is no need for a high pH for this step to happen. According to information submitted by registrants, the truncated B.t. gene in corn produces three more B.t. proteins; it is therefore highly probable that the toxin-like protein of the transgenic corn can also be activated in insects with non-alkaline intestines.* (17-18)

The essence of Petitioners' argument is that the endotoxin produced by *B.t.* corn is closer to the form of the active endotoxin that results from cleavage of a "protoxin" in the gut of susceptible insects. Petitioners assert that it is "highly probable" that the endotoxin produced by transformed corn can be activated in the non-alkaline guts of insects that typically are not susceptible to the toxin precisely because they do not activate the protoxin - thus expanding the susceptible insect host range. Petitioners present no data or evidence to support the proposition that such expansion of susceptible insect host range is "highly probable."

Because the isolated *B.t.* endotoxin genes and proteins in transformed plants usually are "truncated", i.e., of a smaller molecular weight and in "activated" form, theoretically these could be in a more readily toxic form to a wider range of animal life. Therefore EPA treated the *B.t.* proteins expressed by transformed plants as new active ingredients and required submission of new data prior to registering these products. EPA required, *inter alia*, registrants to test the expansion of susceptible insect host range. EPA has not received and is not aware of any data indicating either expansion of

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194 Even if these results were valid, however, if taken at face value, they would support the proposition that there should not be any significant adverse effects on *Collembola* at normal soil concentrations.

susceptible insect host range or harm to the beneficial insects tested.[195] The only change, if present, was to increase the efficacy against the target insect pests. The truncated forms of the protein toxin are also commonly found in the commercially available, microbially produced foliar sprays of *B.t.* and may be the result of inherent proteases expressed during sporulation or natural degradation. Petitioners state that the truncated "toxin-like" protein "only needs a minor step to turn it into an active toxin." This so called "minor step" is trypsin activation which is what activates all the protoxins into active toxins. Thus, there is no real difference between the transformed corn produced protoxin and protoxins normally found in foliar spray products. As for Petitioners' statement that "[i]t seems that there is no need for a high pH for this step to happen," the point is irrelevant. No adverse effects have been seen in any animal, e.g., mammals, that have acidic guts.

As for the three additional *B.t.* proteins reported by registrants, these same degradates of the expressed toxin are found in all the *B.t.* plants seen to date and probably represent the action of inherent plant proteases on the expressed protein. EPA is aware of infrequent, unconfirmed reports of toxicity by the activated endotoxins to insects which are not susceptible to the intact *B.t.* endotoxin. These data have not been confirmed by other studies. None of the data reviewed by EPA confirm a risk to insects not susceptible to the native crystal toxin. To date EPA found no changes in the target insect host range (with or without alkaline guts) as a result of truncation of the *cry* genes in plants. In addition, no adverse effects to a substantial number of individual non-target invertebrate populations in the field attributable to truncated Cry proteins from plants have been reported to date.

(4) *A recent study suggests that transgenic B.t. plants may create serious impacts on non-target organisms that feed on pests exposed to the transgenic toxins.* (18)

Petitioners cite a recent study for the proposition that transgenic *B.t.* plants may create serious impacts on nontarget organisms that feed on pests exposed to the transgenic toxins. The effects of *B.t.* corn on larvae of the beneficial predatory insect, lacewing, cited by Petitioners, stem largely from reports of work by Hilbeck, *et al.* of the Swiss National Science Foundation.[196] EPA recently performed a formal review of the two Swiss laboratory studies on the effects of *B.t.* corn and pure *B.t.*

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195 See the *B.t*. plant-pesticide registration Fact sheets. As discussed above, EPA believes the one study indicating adverse effects to *Collembola* to be an outlier.

196 Hilbeck, A., M. Baumgartner, P.M. Fried, F. Bigler, Effects of *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: *Chrysopidae*), 27(2) Environ. Entomol 480-487 (1998); Hilbeck, A., Moar, W.J., Pusztai-Carey, M., Fillippi, A., and F. Bigler, Toxicity of *Bacillus thuringiensis* CryIAb Toxin to the Predator *Chrysoperla carnea* (Neuroptera: *Chrysopidae*), 27(5) Environ. Entomol 1255-1263 (1998).

corn toxin on lacewing.[197] While the authors report detrimental effects on lacewing larvae from consumption of *B.t.* corn toxin, their data show that lacewing mortality and developmental effects more likely are related to the study diet, not to any potential *B.t.* endotoxin effects. Moreover, even if the reported results are taken at face value, the adverse effects are so slight as to suggest no significant impact on a substantial number of individual beneficial insects in a population in the field.

Hilbeck, *et al.*, report slightly elevated mortality and prolonged development time in lacewing larvae reared on *B.t.* intoxicated prey (the European corn borer - ECB). The experimental design of the study, however, did not permit a distinction between a direct effect due to the *B.t.* toxin on the predator versus an indirect effect of consuming a suboptimal diet consisting only of sick or dying prey that had succumbed to the *B.t.* toxin. The dead or dying prey may have been septicemic (and therefore indirectly toxic), of limited nutritional value, or unpalatable to the lacewing. The lacewing was not given a choice in diet, which it has in a field setting. In nature the lacewing does not rely upon a single food source for development. In addition, the study has a high control mortality (34%, which is indicative of an unhealthy test system) and no prey consumption data. Also, there was no control with the purified *B.t.* endotoxin. Generally, the findings are inconclusive and the laboratory report results are not directly transferable to the field use setting. The authors conclude that "...trials investigating predation efficiency and predator performance under field conditions are necessary before conclusions regarding the potential ecological relevance of the results presented in our paper can be drawn."[198] In addition, all available Agency in-house and published field data do not show significant detrimental effects due to *B.t.* endotoxin on the lacewing.[199]

Moreover, the authors subsequently reassessed the results of the study cited by Petitioners on reproductive effects on beneficial non-target organisms exposed to *B.t.* corn in the laboratory.[200] According to Hilbeck, there are no significant reproductive effects from *B.t.* corn toxin. The authors thus conclude that "[s]urviving, unaffected *C. carnea* developed at rates similar to those in the untreated control" and "[f]rom this, we conclude that total developmental time until adult eclosion is not an appropriate parameter for detecting Cry1Ab toxin effects."[201]

The second study cited by Petitioners <u>used</u> defined quantities of pure *B.t.* toxin and there was significant mortality only in an *artificial* diet test group, and no significant mortality when the artificial

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197 The reviews (DP Barcode D250457 and D236803) have been placed in the Special Docket.

198 Hilbeck, <u>et al.</u>, 27(5) Environ. Entomol 1255-1263.

199 *B.t.* plant-pesticide registration Fact Sheets are available in the Special Docket.

200 Hilbeck, <u>et al.</u>, 27(2) Environ. Entomol 480-487.

201 Hilbeck, <u>et al.</u>, 27(5) Environ. Entomol 1255-1263.

diet was supplemented with *E. kuehniella* eggs (a natural diet).[202] Therefore, this study does not demonstrate any adverse effects to lacewing larvae under simulated field feeding habits where the lacewing larvae have a choice of natural diet in the field. Moreover, in this study, the concentration of pure Cry protein to which the larvae were exposed was massive (100 micro gm/ml of diet) and continuous, and therefore not reflective of Cry1Ab exposures that may occur under field conditions - either by exposure to plant tissues, pollen or by consumption of exposed prey species, such as ECB larvae. The dosage used in these studies is at least 30 times that found in most corn tissues in the field. Also, since in the field setting the lacewing larvae have a choice of other insects or eggs to feed on, field exposure will be intermittent, rather than continuous. Furthermore, in high-dose *B.t.* corn fields intoxicated insects such as the ECB will not be available to the lacewings, since the ECB will be practically eliminated early by the *B.t.* toxin in corn plants.[203] In addition, any surviving ECB larvae would normally be within the corn plant most of their larval life and not available for consumption by chrysopids.[204]

As noted in EPA's review of the first study, the lack of quantitation of *B.t.* consumption by the larvae makes it impossible to determine correlation between exposure to *B.t.* and the observed responses. No data were presented to show the amount of prey consumed by each test group to make an independent assessment of unpalatability and sick prey effects that might be the result of food avoidance. The same is also true of the second study, in that it is not reported how much of a reduction in consumption of *B.t.* toxin occurred in the replicates receiving a choice in diet. It is clear in this study also that there is a detrimental effect of the artificial study diet because data are presented that show an increase in mortality and development time in larvae reared exclusively on an artificial diet. Thus, the results of these studies do not support the conclusion that the *B.t.* toxin was directly responsible for the observed differences in lacewing mortalities.

Environmental influences were also not considered in the speculation that *B.t.* corn may pose a risk to beneficial insects. In a field setting it is highly improbable that lacewing larvae will mature exclusively on a diet of prey larvae that have been exposed to *B.t.* endotoxin. Therefore it is highly unlikely that in the field the lacewings, or other beneficial insects, will ingest the amounts of *B.t.* that the larvae were forced to consume in the laboratory study (i.e. there is a very low field exposure to the *B.t.* toxin). The reported laboratory findings are not representative of the feeding environment by predatory insects in the open ecosystem, nor is the exposure to *B*.t. endotoxin consistent with exposure that would be expected in the field.

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202 Id.

203 The first instars die as soon as they start eating *B.t.* corn tissue.

204 ECB larvae live within the corn stalk, not on stalk surface.

In general the reported laboratory findings do not show significant detrimental effects and do not provide data that show a risk to beneficial insects in a field use situation. The author, A. Hilbeck, agrees with this by stating that "...trials investigating predation efficiency and predator performance under field conditions are necessary before conclusions regarding the potential ecological relevance of the results presented in our paper can be drawn."[205] Moreover, there are published field studies on the effects of *B.t.* crops on insect predators showing no significant differences in the density of beneficial insects.[206] These published field testing results and field test data submitted to EPA show minimal to undetectable changes in the beneficial insect abundance.[207] Some actually report the densities of predatory and non-target insects as generally higher on transgenic than non-transgenic crops. To date the available field test data show that compared to crops treated with conventional chemical pesticides, the transgenic crops have no detrimental effect on a substantial number of individuals in beneficial insect populations.

(5) *Decomposition of plant after harvest may result in the accumulation of B.t. toxins at soil concentrations high enough to constitute a hazard to non-target organisms, such as beneficial insects (e.g., pollinators, parasites, and predators of insect pests) and other animal classes. This could result in the selection and enrichment of toxin-resistant insects.* (18)

In reviewing existing data, there are no studies or data indicating that *B.t.* crops result in accumulation of *B.t.* endotoxin in soils. In fact, data developed for *B.t.* crop registrations show that this is not the case. The publications cited in the Petition in support of speculative harm to the soil ecology (ref. 50, p. 18) were a result of work that was initiated and funded by the Agency. Starting in the late 1980s, in anticipation of applications for registration of *B.t.* crops, EPA's Office of Research and Development performed and funded studies on the fate of *B.t.* endotoxins in soil. The results of this work were taken into consideration by the Agency in the registration decisions of *B.t.* plant-pesticides. To address the potential for ecological effects from plant material containing expressed *B.t.* endotoxins, EPA has required submission of soil degradation studies on vegetation incorporated into the soil prior

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205 Hilbeck, et al., 27(5) Environ. Entomol 1255-1263.

206 Fitt, G.P., Martes, C.L. and Llewellyn, D.L., Field evaluation and potential ecological impact of transgenic cottons in Australia, 4 Biocontrol Sci.Tech. 535 (1994); Orr, D.B., and D.A. Landis, Oviposition of European Corn Borer (Lepidoptera: *Pyralidae*) and Impact of natural Enemy Populations in Transgenic Versus Isogenic corn, 90(4) J. Econ. Entomol. 905 (1997); Pilcher, C.D., M.E. Rice, J.J. Obrycki and L.C. Lewis, Field and Laboratory Evaluations of Transgenic *Bacillus thuringiensis* Corn on Secondary lepidopteran pests (Lepidoptera: *Noctuidae*), 90(2) J. Econ. Entomol. 669-678 (1997).

207 These data are discussed in the *B.t.* plant-pesticide registration Fact Sheets and are included in the Special Docket.

to registration of each *B.t.* crop. In all cases the registration data showed that the *B.t.* toxin was undetectable by target insect bioassay after 40 days or less, with a soil half-life in the range of 3-7 days.[208]

There is no evidence that *B.t.* endotoxins partition into the soil from *B.t.* crops, or that *B.t.* proteins are in a form that cannot be readily degraded by soil biota. In response to reports of potential harm to soil invertebrates, EPA required data on effects on representative soil beneficial species [earthworm and the springtail (*Collembola*)] and beneficial predatory above-ground species (usually honey bee/larvae, green lacewing larvae, ladybird beetle and parasitic wasp and at times other species, such as predatory mites) for all of the plant-pesticides registered to date.[209] In none of these studies, was toxicity seen at *B.t.* doses much higher than what would be expected to be in the soil as a result of *B.t.* crop cultivation.

Petitioners' assertion that "*[d]ecomposition of plant after harvest may result in the accumulation of B.t. toxins at soil concentrations high enough to constitute a hazard to non-target organisms, such as beneficial insects (e.g., pollinators, parasites, and predators of insect pests) and other animal classes*" is speculative and based on limited laboratory studies which are not applicable to actual field conditions. Soils are the natural habitat of all *B.t.* species; therefore *B.t.* is already naturally present during the crop growing season and is naturally "exerting selective pressure" (if there is any), especially so in soils after application of conventional *B.t.* sprays. Moreover, the extent of the *Bacillus thuringiensis* bacteria's range extends throughout the United States into all areas where *B.t.* crops may be planted. *B.t.* spores do persist in the soil and are constantly available for ingestion by all soil invertebrates and the toxin is available for binding to clay.

Moreover, recent work by Stotzky shows that binding to clay and humic acid soils and subsequent insecticidal activity (>6 months) is strong only at a low pH (4.9 - 5.1).[210] Agricultural soils are limed when necessary to be at a pH range of 5.8 - 7.3.[211] Stotzky showed that, within that pH

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208 These data are discussed in the *B.t.* plant-pesticide registration Fact Sheets and are included in the Special Docket.

209 These data are discussed in the *B.t.* plant-pesticide registration Fact Sheets and are included in the Special Docket.

210 Crecchio, C. and G. Stotzky, Insecticidal Activity and biodegredation of the Toxin from *Bacillus thuringiensis* subspecies *kurstaki* bound to humic acids in soil, 30(4) Soil Biol. Biochem. 463 (1998).

211 R.M. Atlas and R. Bartha, Microbial Ecology, p. 354 (Benjamin/Cummings Publ. Co., 3rd ed. 1993).

range, *B.t.* endotoxins are released from the clay and degraded by soil microbes.[212] Stotzky's data show that at a pH of 5.8 - 7.3 the insecticidal activity was reduced to 35 days duration.[213] This is in the range shown by the data developed for each *B.t.* crop registration.

(6) *The addition of purified bacterial B.t.-protoxins to soil did not cause any detectable effects on examined soil bacteria, fungi and other microorganisms. In contrast, the addition of some transgenic B.t. cotton plants frequently caused significant although transient stimulation of culturable, aerobic bacteria and fungal populations.* (18)

Petitioners do not assert that the transient alteration of the soil microbe ratios constitutes an adverse effect of any sort. EPA believes that the shift in the ratios of soil invertebrates or culturable aerobic bacteria and fungi upon addition of *B.t.* cotton plants to the soil (Petitioners ref. 51) is indicative of, and consistent with natural fluctuations in soil invertebrate/microbial abundance which result from constant changes in the availability of organic matter, type of organic matter, temperature, moisture content, resultant pH fluctuations, inhibitory microbial metabolite concentrations, etc. Except as it applies to soil fertility, which is dependent on the presence of organic matter, essential minerals, adequate moisture and proper pH, there is no practical agricultural significance to this ongoing fluctuation in the relative species composition of various soil micro-ecosystems. The research cited by Petitioners was conducted with *B.t.* cotton in laboratory microcosms by EPA's Office of Research and Development. These findings were taken into consideration by EPA in its risk assessments which preceded the registration of *B.t.* plant-pesticides.[214]

(7) *Purified B.t. toxins, as produced by transgenic plants, continue to be active for a surprisingly long time in some soils and keep their toxic effects. Active B.t. toxins in the soil have been found even nine months after the toxins had been released. Toxins are bound on soil constituents (e.g., clay-particles) and are thus protected against decomposition and microbial degradation. B.t. protoxins within bacteria, on the other hand, decompose on average twice to three times as quickly as do the active toxins.* (18-19)

EPA does not believe that Petitioners' assertions of prolonged environmental persistence of transgenic *B.t.* constitute an actual or potential adverse effect. EPA is aware that there are reports in the literature suggesting that transgenic *B.t.* proteins can be bound to soil particles and resist

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212 Crecchio, et al., 30(4) Soil Biol. Biochem. 463-470.

213 Id.

214 See *B.t.* plant-pesticide Fact Sheets available in the Special Docket.

degradation.[215] EPA, however, has received data from registrants prior to registration of transgenic *B.t.* endotoxins that indicates the transgenic proteins degrade when added to soil, based upon bioassay with a susceptible insect species.[216]

In general, the stability of *B.t.* endotoxins in soil does not appear to be any different from that expected for any protein or DNA subject to soil binding and microbial degradation. The claim that soil-bound *B.t.* endotoxins from plants will persist and have additive adverse effects on numerous non-target organisms is unsupported. Moreover, data has been submitted to EPA indicating that, based on the bioactivity of *B.t.* endotoxins added to soil, the endotoxins remain available and active with a half-life of five days.[217]

The assertion that *B.t.* endotoxins bound to clay and humic acid soils are not available to microbial degradation and thus persist and accumulate in the environment is based on laboratory studies of *B.t.* **spray** products - **not** transgenic *B.t.* endotoxin. The studies performed by Stotzky, *et al.* were in laboratory clay and clay enriched soils using purified *B.t.* toxins that were not bound to plant tissues, as they are in a field setting.[218] All of Stotzky's reported work used free, purified *B.t.* toxin, whereas *B.t.* toxin in the field bound to decaying plant tissue is not available for immediate contact with clays in

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215 Crecchio, C. and G. Stotzky, Insecticidal Activity and biodegredation of the Toxin from *Bacillus thuringiensis* subspecies *kurstaki* bound to humic acids in soil, 30(4) Soil Biol. Biochem. 463-470 (1998); Koskella, J. and G. Stotzky, Microbial Utilization of Free and Clay-Bound Insecticidal Toxins from *Bacillus thuringiensis* and their Retention of Insecticidal Activity after Incubation with Microbes, 63(9) Appl. Environ. Microbiol. 3561-3568 (1997); Tapp, H. and G. Stotzky, Insecticidal Activity of the Toxins from *Bacillus thuringiensis* subspecies *kurstaki* and *tenebrionis* Adsorbed an Bound on Pure and Soil Clays, 61(5) Appl. Environ. Microbiol. 1786-1790 (1995); Tapp, H. and G. Stotzky, Dot Blot Enzyme linked Immunosorbent Assay for Monitoring the Fate of Insecticidal Toxins from *Bacillus thuringiensis* in soil, 61(2) Appl. Environ. Microbiol. 602-609 (1995); Tapp, H. and G. Stotzky, Persistence of the Insecticidal Toxin from *Bacillus thuringiensis* subspecies *kurstaki* in soil, 30(4) Soil Biol. Biochem. 471-476 (1998); Venkateswerlu, G., and G. Stotzky, Binding of Protoxin and Toxin Proteins of *Bacillus thuringiensis* subsp. *kurstaki* on Clay Minerals 25 Current Microbiology 225-223 (1992).

216 These data are discussed in the *B.t.* plant-pesticide registration Fact Sheets and are included in the Special Docket.

217 These data are discussed in the *B.t.* plant-pesticide registration Fact Sheets and are included in the Special Docket.

218 footnote191, *supra*.

the soil. Thus, this work does not support the proposition that transgenic *B.t.* endotoxins derived from plant matter are persistent and active for an extraordinary time frame.

l. Petitioners' arguments do not demonstrate, nor does EPA have reason to believe, that *B.t.* plant-pesticide registrations cause unreasonable adverse effects on the environment thus requiring cancellation of all such registrations under FIFRA

In conclusion, EPA does not believe that the data and information provided by Petitioners, when viewed in conjunction with all the data and information available to EPA, raise sufficient questions concerning whether these registrations may cause unreasonable adverse effects on the environment to justify cancellation proceedings under FIFRA Section 6. As discussed in Appendix A, EPA may seek to cancel a pesticide registration upon a determination that the widespread and commonly recognized use of a pesticide "generally causes unreasonable adverse effects on the environment."[219] Cancellation proceedings under FIFRA Section 6 may entail, at the registrant's discretion, a highly involved, on the record, hearing before an administrative law judge, who may request a report from the National Academy of Sciences on questions of scientific fact. At present, EPA does not believe that the current *B.t.* plant-pesticide registrations cause unreasonable adverse effects on the environment. EPA has imposed reasonable, science-based IRM requirements on these registrations that, based on the current data and information, should mitigate the development of pest resistance. EPA does not believe that the registered *B.t.* plant-pesticides will result in adverse ecological effects, either by outcrossing to weedy plant relatives, or by adversely affecting non-target beneficial insects. Moreover, EPA does not believe that the potential risk of adverse effects to man or the environment from use of these registered plant-pesticides is sufficient to constitute an unreasonable adverse effect, when taking into account the benefits of the use of these plant-pesticides.[220] Thus, EPA does not agree that the registration of *B.t.* plant-pesticides has created unreasonable adverse effect on the environment, or will result in significant negative economic, social and environmental impacts.

To ensure that adverse effects related to registered *B.t.* plant-pesticides do not occur over the long-term, EPA is comprehensively reassessing the expiring *B.t.* plant pesticide registrations and pest management resistance requirements, to ensure public health and environmental protection. This process will be scientifically-based and provide increased opportunities for public comment and participation on both EPA's scientific risk assessment and EPA's risk management proposals. The comprehensive reassessment will include (1) consideration by EPA of all currently available information on the risks and benefits of *B.t.* corn, cotton, and potato plant-pesticides; (2) development by EPA scientists of an updated risk assessment for *B.t.* plant-pesticides; (3) outside scientific peer review of

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219 7 U.S.C. § 136d(b).

220 A summary of the benefits assessment for each *B.t.* plant-pesticide is found in the *B.t.* plant-pesticide Fact Sheets, which may be found in the Special Docket for this action.

EPA's risk assessment by the FIFRA Scientific Advisory Panel (SAP); (4) public comment on EPA's risk assessment; and (5) other opportunities for public involvement. EPA fully intends to reach a decision on the existing registrations in a timely fashion. This comprehensive reassessment will guide EPA in determining how it will handle future *B.t.* plant-pesticide applications for registration.

2. Currently Available Data and Information Do Not Warrant Initiation of a Special Review for Each of the Challenged Registrations

Petitioners assert that, in accordance with EPA's regulations, "*the adverse reproductive effects and the risk to the environment caused by the registration of genetically engineered plants expressing B.t. warrant initiation of special review of all registrations.*"[221] This is incorrect.

Special Review is a process that EPA utilizes to determine "whether the use of a pesticide poses unreasonable adverse effects to humans or the environment."[222] EPA conducts Special Review in accordance with the provisions of Section 3(c)(8) of FIFRA:

> Notwithstanding any other provision of this Act, the Administrator may not initiate a public interim administrative review process to develop a risk-benefit evaluation of the ingredients of a pesticide or any of its uses prior to initiating a formal action to cancel, suspend, or deny registration of such pesticide, required under this Act, unless such interim administrative process is based on a validated test or other significant evidence raising prudent concerns of unreasonable adverse risk to man or to the environment. Notice of the definition of the terms "validated test" and "other significant evidence" as used herein shall be published by the Administrator in the Federal Register.[223]

EPA defines "validated test" as "a test determined by the Agency to have been conducted and evaluated in a manner consistent with accepted scientific procedures."[224] The term "other significant evidence" is defined as "factually significant information that relates to the uses of the pesticide and their adverse risk to man or to the environment, but does not include evidence based only on misuse of the pesticide unless such misuse is widespread and commonly recognized practice."[225]

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221 Petition at 23.

222 Special Reviews of Pesticides; Criteria and Procedures, 50 Fed. Reg. 49003 (Nov. 27, 1985).

223 7 U.S.C. § 136a(c)(8).

224 40 C.F.R. § 153.3(i).

225 40 C.F.R. § 154.3(e).

EPA believes that Congress intended in enacting Section 3(c)(8) that EPA "have a reasonable scientific basis for its risk concerns prior to initiating the public Special Review process."[226] EPA evaluates specific criteria in making a determination whether a reasonable scientific basis exists for initiation of a Special Review. The criteria for determining whether a reasonable scientific basis exists to conduct a Special Review of a pesticide use or uses are (1) may pose a risk of serious acute injury to humans or domestic animals; (2) may pose a risk of inducing in humans an oncogenic, heritable genetic, teratogenic, fetotoxic, reproductive effect, or a chronic or delayed toxic effect; (3) may result in residues in the environment of non-target organisms at levels which equal or exceed concentrations acutely or chronically toxic to such organisms, or at levels which produce adverse reproductive effects in such organisms, as determined from tests conducted on representative species or from other appropriate data; (4) may pose a risk to the continued existence of any endangered or threatened species designated by the Secretary of the Interior or the Secretary of Commerce under the Endangered Species Act of 1973, as amended; (5) may result in the destruction or other adverse modification of any habitat designated by the Secretary of the Interior or the Secretary of Commerce under the Endangered Species Act as a critical habitat for any endangered or threatened species; (6) may otherwise pose a risk to humans or to the environment which is of sufficient magnitude to merit a determination whether the use of the pesticide product offers offsetting social, economic, and environmental benefits that justify initial or continued registration.[227]

In promulgating these criteria, EPA intended to retain substantial discretion in determining whether and when to conduct a Special Review.[228] Moreover, even if one of the Special Review triggers is met, EPA is not required to initiate a Special Review automatically. Special Review is a discretionary tool that the Agency can utilize, when it is appropriate.

Petitioners have cited certain data and information in support of their assertion that initiation of Special Review of the registrations of *B.t.* plant-pesticides is warranted. EPA has evaluated these data and information in the context of (1) Congress' intent that EPA "have a reasonable scientific basis for its risk concerns prior to initiating the public Special Review process," and (2) the Section 154.7 criteria promulgated by EPA to effectuate Congress' intent. The data and information presented by Petitioners do not meet the regulatory threshold for initiation of a Special Review of the *B.t.* plant-pesticide registrations.

a. <u>Responses to Petitioners' Specific Arguments</u>

(1) *Adverse reproductive effects should trigger special review*. (23)

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226 50 Fed. Reg. at 49006/3.

227 40 C.F.R. § 154.7.

228 50 Fed. Reg. at 49007.

Petitioners argue that, in accordance with 40 C.F.R. § 154.7(a)(3), initiation of Special Review is warranted because the registration of *B.t.* plant-pesticides may result in residues in the environment impacting non-target organisms that produce adverse reproductive effects on such organisms.[229] As discussed in greater detail in section II.A.1.j., EPA does not believe that the current relevant scientific information and data support a conclusion that cultivation of transgenic plants expressing *B.t.* plant-pesticides may have adverse impacts to non-target organisms - including adverse reproductive effects. Moreover, EPA believes that evidence supports a conclusion that non-target beneficial organisms are maintained or enhanced in fields where *B.t.* plant-pesticides are used. See previous discussion in section II.A.1.k.

At present, EPA is aware of no identified significant adverse effects of *B.t.* endotoxins to non-target beneficial organism populations in the field, whether they are pest parasites, pest predators, or pollinators. Published field testing results and field test data submitted to EPA show minimal to undetectable changes in the beneficial insect populations. Densities of predatory and non-target insects are generally higher on *B.t.* plant-pesticide crops than non-*B.t.* crops solely because the *B.t.* crops are not subjected to spraying with nonspecific pesticides. Thus, EPA does not believe that the currently available information indicates a potential for adverse impacts to non-target organisms sufficient for EPA to have a reasonable scientific basis that sufficient risk exists to justify initiation of a public Special Review process.

(2) *Risks to the environment of sufficient magnitude should trigger special review.*

Petitioners argue that, in accordance with 40 C.F.R. § 154.7(a)(6), initiation of Special Review is warranted because the registration of genetically engineered plants expressing *B.t.* may result in cumulative environmental impacts of sufficient magnitude to trigger special review.[230] Petitioners' arguments are based on speculation that (i) development of *B.t.* tolerant pests would trigger the use of more synthetic pesticides to substitute for foliar *B.t.* sprays render inefficacious against the *B.t.*-resistant pests,[231] and (ii) gene flow to weedy relatives and (iii) harm to non-targets will create environmental impacts of sufficient magnitude to trigger special review.[232]

EPA concludes that Petitioners' speculations that development of pests resistant to *B.t.* plant-pesticides will lead to increased use of synthetic chemicals do not to constitute a reasonable scientific basis that sufficient risk exists to justify initiation of a public Special Review process. As discussed

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229 Petition at 23.

230 Id.

231 Petition at 23-24.

232 Petition at 24.

above in section II.A.1.d., EPA has taken extensive and unprecedented measures to have resistance management plans in place to mitigate the development of pests resistant to *B.t.* crops. Moreover, EPA has taken action to strengthen these requirements when scientific evidence pointed to a need for stronger measures. EPA will continue to require stringent IRM plans, as supported by the scientific evidence to mitigate the risk of resistance development.

EPA is not aware of any data or information, nor have Petitioners presented any data or information, indicating that any organisms resistant to *B.t.* have developed as a result of cultivation of crops expressing registered *B.t.* plant-pesticides since the first such registrations in 1995. EPA's substantial efforts to mitigate the development of pests resistant to *B.t.* endotoxins are ongoing and, as discussed above, EPA will conduct a public scientific review process in 2000, in which all available data and information relevant to pest resistance management will be evaluated to develop IRM terms and conditions for future *B.t.* corn and cotton plant-pesticide registrations that reflect the current scientific understanding of the development of pest resistance. Finally, EPA believes that it would be somewhat anomalous for the Agency to initiate a Special Review on the basis of speculation that *B.t.* plant-pesticides may lead to pest resistance, which may lead to increased use of synthetic chemical pesticides, when, in fact, there are actual data demonstrating that cultivation of these crops have led to <u>decreases</u> in the amount of synthetic chemical pesticides applied. [233] In effect, EPA is being asked to initiate a Special Review to determine whether to forego actual existing positive environmental effects, solely on the basis of speculative future impacts.

EPA does not agree that speculation that gene flow from transgenic *B.t.* crops to weedy relatives constitutes a reasonable scientific basis that sufficient risk exists to justify initiation of a public Special Review process. As discussed in great detail above in section II.A.1.g., EPA has investigated the potential outcrossing of *B.t.* toxins expressed in potato, corn, and cotton to wild or weedy relatives in the United States, its possessions or territories. EPA has determined that the possibility of outcrossing to wild or weedy relatives is unlikely to occur between *B.t.* corn because domestic corn has no wild or weedy relatives in the United States with which there exists a substantial risk of outcrossing and spread. Similarly, EPA has determined that B.t. potato varieties are unlikely to outcross to wild or weedy relatives because they are geographically and temporally isolated from wild or weedy relatives for which they could conceivably be sexually compatible. EPA has determined that it is possible for outcrossing to occur between *B.t.* cotton and its wild or weedy relatives in the United States, its possessions or territories. Therefore, EPA imposed strict sales and distribution restrictions as terms and conditions of the *B.t.* cotton registrations to prevent the possibility of outcrossing occurring with wild or weedy relatives. These containment measures prevent the movement of the B.t. cotton to wild or weedy cotton relatives that exist in Hawaii and Florida.

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[233] Gianessi, L.P., J. E. Carpenter, <u>Agricultural Biotechnology: Insect Control Benefits</u>, National Center for Food and Agricultural Policy, Washington D.C. (1999).

For the reasons stated here and in greater detail in the referenced text, EPA does not believe that a reasonable scientific basis exists to conclude that the registered *B.t.* plant-pesticides may pose sufficient risks to meet any of the risk triggers set forth at 40 C.F.R. § 154.7 for initiation of Special Review.

B. Response to Petitioners' NEPA Arguments

1. EPA is not Obligated to Conduct a NEPA Analysis of Its *B.t.* Plant-Pesticide Registrations

Petitioners argue that EPA's registrations of *B.t.* plant-pesticides require a programmatic environmental impact statement (PEIS) under the National Environmental Policy Act (NEPA).[234] Petitioners are incorrect as a matter of law. Courts have consistently held that NEPA does not apply to the registration of pesticides under FIFRA because the procedures established in FIFRA are the functional equivalent of NEPA.[235] Therefore, EPA is not required to prepare an Environmental Assessment ("EA"), an Environmental Impact Statement ("EIS"), or a Programmatic EIS ("PEIS") when registering pesticides under FIFRA.

The challenged registrations fall squarely within the functional equivalence exemption to NEPA. FIFRA, by its terms, necessitated a complete review of the environmental risks and benefits of these applications for pesticide registration, including cumulative impacts of the registrations. Further, pursuant to FIFRA, EPA's regulations provide the opportunity for public comment on applications for pesticide registration for new active ingredients or new uses.[236] Where, as here, EPA has "engaged primarily in an examination of environmental questions, where substantive and procedural standards ensure full and adequate consideration of environmental issues, then formal compliance with NEPA is

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234 42 U.S.C. §§ 4321-4370d. Petition at 24.

235 Environmental Defense Fund v. EPA, 489 F.2d 1247, 1256-57 (D.C. Cir. 1973) (the court analyzed the requirements of FIFRA and NEPA and decided that NEPA does not apply); Environmental Defense Fund v. Blum, 458 F. Supp. 650, 661-62 (D.D.C. 1978). See also Foundation on Economic Trends v. Thomas, 637 F. Supp. 25, 28-29 (D.D.C. 1986); Wyoming v. Hathaway, 525 F.2d 66, 71-73 (10th Cir. 1975) (EPA need not prepare formal EIS prior to suspension of a pesticide when substantial equivalent was performed). Other courts have reached the same conclusion, reasoning that Congress did not intend NEPA to apply to EPA's pesticide registrations under FIFRA. Merrell v. Thomas, 807 F.2d 776, 781 (9th Cir. 1986).

236 See 7 U.S.C. § 136a(c)(4).

not necessary, but functional compliance is sufficient."[237] The procedures undertaken by EPA clearly meet NEPA's twin aims of requiring agencies to consider the environmental impacts of their actions and informing the public of the consideration of those impacts.[238] To "require a 'statement' [PEIS], in addition to a decision [under FIFRA] setting forth the same considerations would be legalism carried to the extreme."[239]

To the extent Petitioners are attempting to circumvent the functional equivalence exemption by arguing that the *B.t.* plant-pesticide registrations must be considered together as a "program" for which a programmatic EIS (PEIS) is required, that argument must be rejected.[240] In Lyng, the D.C. Circuit considered whether a series of seemingly related agency actions in fact constituted a "program" requiring a PEIS. The Court held that a "commonality of objective" and other "rudimentary similarities" do not suffice to bind a series of individual decisions into either a "program" or "major federal action" for which a PEIS would be required.[241] The Court recognized that where the agency's decisions are "discrete and independent in nature," the "[a]pproval of one project does not insure approval of technologically similar projects," and the agency's decisions are "largely reactive . . . to respond to the needs of . . . [the agency], other regulatory agencies user groups and consumers," the actions do not require a PEIS.[242]

Each of those factors applies here, as well. While the *B.t.* registrations share a "rudimentary similarity" in that each addresses a *B.t.* plant-pesticide, the Agency's decisions on the individual registrations are discrete and independent - an approval of one *B.t.* plant-pesticide registration application does not insure approval on any other.[243] Moreover, EPA's pesticide registration decisions, unlike those of the agency in Lyng, are completely reactive. EPA does not develop pesticides, and it only considers pesticides for registration upon receipt of a complete application from a registration applicant.[244] Thus, the plant-pesticide registrations do not constitute a "program" to which NEPA applies.

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237 EDF v. EPA, 489 F.2d at 1257.

238 Baltimore Gas & Electric Co. v. NRDC, 462 U.S. 87, 97 (1983).

239 EDF. v. EPA, 489 F.2d at 1256.

240 Foundation on Economic Trends v. Lyng, 817 F.2d 882 (D.C. Cir. 1987).

241 Id. at 884-85.

242 Id.

243 See 40 C.F.R. §§ 152.112, 152.113, and 152.114.

244 Id. §§ 152.104, 152.105.

2. NEPA Is Not Applicable Because No "New" Regulatory Plant Pesticide Program Exists.

Petitioners assert that EPA's registration of plant-pesticides is a "new" regulatory program that began with a Notice of Proposed Rulemaking published at 59 Fed. Reg. 60519 (Nov. 23, 1994), and for which Petitioners argue a PEIS under NEPA is required. Contrary to Petitioners' assertions, no "new" regulatory plant pesticide program exists and to the extent this is an attempt to circumvent the functional equivalence exemption it too must fail.

The proposed rule Petitioners cite is not applicable to *B.t.* plant-pesticides and therefore is irrelevant to Petitioners' assertions. The proposed rule, published under the authority of FIFRA section 25(b), proposes to exempt certain categories of plant-pesticides from specific regulatory requirements. None of these exemptions would apply to, or otherwise affect, the *B.t.* registrations that are the subject of this petition. The proposed rule does not establish a "new " *B.t.* pesticide registration program nor does it alter in any way the existing regulatory structure. To the contrary, at the time the proposed rule was published, EPA clarified that unless and until the Agency did undertake rulemaking with regard to plant-pesticides, "existing regulations (e.g., 40 CFR Parts 156, 158, and 172) will be used as the basis for plant-pesticide regulatory procedures."[245] Petitioners' assertion that the proposed rule is a "new" regulatory program that changed EPA's standards and procedures for acting on pesticide registrations must be rejected.

3. Even If the Proposed Rules Did Constitute a Program, the Functional Equivalence Exemption Would Apply

Even assuming, arguendo, that a "new "program exists, the proposed rule the Petitioners allege makes up the "program" is subject to a process that is the functional equivalent of the NEPA process. As is the case with NEPA, consideration of the proposed rule requires EPA to "engage[] primarily in an examination of environmental questions."[246] The rulemaking process also includes "substantive and procedural standards [that] ensure full and adequate consideration of environmental issues."[247] Accordingly, formal compliance with NEPA is not necessary and instead, "functional compliance" is sufficient.[248] Here functional compliance is provided because the pesticide registration process achieves the twin aims of NEPA.

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245 59 Fed. Reg. 60496, 60507 (1994).

246 Environmental Defense Fund v. EPA, 489 F.2d 1247, 1256.

247 Id.

248 Id.

The first of NEPA's twin aims is to ensure that agencies consider the environmental consequences of their actions.[249] FIFRA, by its terms, necessitates a comprehensive review of the environmental impacts of the proposed rules such as those that may be incident to the proposed registration exemptions. The second of NEPA's twin aims is to inform the public.[250] FIFRA ensures the public is informed of the environmental effects of EPA's proposed rules and the Administrative Procedure Act (APA) provides the public with an opportunity to comment on them.

The APA requires procedures under which the public is informed of proposed rules and given an opportunity to comment on them.[251] The effectiveness of the APA's notice and comment procedures is enhanced by FIFRA's requirements for the review and comment of the proposed rules. Prior to publishing a proposed rule in the Federal Register, FIFRA requires EPA to submit the proposed rule to a scientific advisory panel "for comment as to the impact on health and the environment of the [proposed] action."[252] The comments, evaluations, and recommendations of the advisory panel, and the Administrator's response to those comments, are then published in the Federal Register, where they are available to inform public comment during the APA rulemaking procedures.[253] These steps have been taken for the proposed rule cited by Petitioners.[254]

To accept Petitioners' assertions would undermine both the purpose and effect of the functional equivalence exemption. Here, where (1) the registrations have already been the subject of a comprehensive review which, indisputably, is the functional equivalent of NEPA; and (2) where any procedures that may be established if EPA at some time in the future promulgates its long-proposed rule, have, by operation of law, been similarly comprehensively reviewed, applying NEPA to the EPA's challenged actions would be wasteful and redundant.

Therefore, for the reasons set forth above, EPA denies Petitioners' request that it conduct a PEIS of *B.t.* plant-pesticides.

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249 Baltimore Gas & Elec. Co. v. NRDC, 462 U.S. 87, 97 (1983).

250 Id.

251 5 U.S.C. 553.

252 7 U.S.C. 136w(d)(1).

253 Id.

254 See 59 Fed. Reg. 60519.

C. Petitioners' Administrative Procedure Act Arguments

1. Standard of Review Under Section 10(e) of the Administrative Procedure Act

Petitioners argue that EPA has registered *B.t.* plant-pesticides "without adequately developing and/or assessing the adequacy of RMP," and that EPA's actions are "arbitrary, capricious, and an abuse of discretion."[255] Although Petitioners present these arguments in a section captioned "The Administrative Procedure Act," Petitioners nowhere in this section (or anywhere else in the Petition) actually discuss the Administrative Procedure Act, or how it relates to their claims. Therefore, in the absence of direction from Petitioners as to how their arguments purport to implicate the APA, EPA assumes, for purposes of this response, that Petitioners are basing their arguments on Section 10(e) of the APA, which provides for judicial redress in circumstances of unlawful agency action. Section 10(e) of the APA provides, in pertinent part:

> To the extent necessary to decision and when presented, the reviewing court shall decide all relevant questions of law, interpret constitutional and statutory provisions, and determine the meaning or applicability of the terms of an agency action. The reviewing court shall - . . . (2) hold unlawful and set aside agency action, findings, and conclusions found to be - (A) arbitrary, capricious, an abuse of discretion, or otherwise not in accordance with law; . . . [256]

Section 10(a) of the APA provides that "[a] person suffering legal wrong because of agency action, or adversely affected or aggrieved by agency action within the meaning of a relevant statute, is entitled to judicial review thereof.[257] Notwithstanding Petitioners' failure to explain the relevance of their "APA" arguments, the Petition indicates that, "[i]n the absence of affirmative response" to the relief requested in the Petition, Petitioners "will be compelled to consider litigation in order to achieve the agency actions requested."[258]

EPA disagrees that its *B.t.* plant-pesticide registration actions are arbitrary, capricious, or an abuse of discretion as that phrase is interpreted under the APA. EPA believes that relevant caselaw interpreting Section 10(e)(A)(2) of the APA demonstrates that its actions were, and continue to be,

255 Petition at 27-28.

256 5 U.S.C. 706(2)(A).

257 5 U.S.C. § 702.

258 See Petition at 33.

well within its discretionary authority as the Agency entrusted by Congress to implement the complex requirements of FIFRA.

The Court of Appeals for the District of Columbia Circuit (hereafter, D.C. Circuit) has held that, in the case of adjudicatory proceedings, e.g., licensing or permit actions, courts "must determine whether the agency has articulated rational connection between the facts found and the choice made; '[w]e may reverse only if the agency's decision is not supported by substantial evidence, or the agency has made clear error in judgment.'"[259] As is discussed extensively within this Response document, all of Petitioners' arguments are based on preliminary, speculative data or information that they believe are sufficient to constitute reasonable bases for EPA to take the rather drastic regulatory actions that they have requested. Petitioners are mistaken, however, for neither the data and information presented in the Petition, nor additional data and information received by EPA since the Petition was served on September 16, 1997, constitute sufficient basis to demonstrate that EPA's registration decisions regarding *B.t.* plant-pesticides were not supported by substantial evidence or were manifestations of clear errors in judgment. Petitioners have not demonstrated that any factual basis exists to substantiate either that EPA's *B.t.* plant-pesticide registration decisions were (1) "not supported by substantial evidence" or (2) a "clear error in judgment." Thus, EPA believes that there is no basis either for a claim or finding that the *B.t.* plant-pesticide registrations were arbitrary, capricious, or an abuse of the Agency's broad discretion to implement FIFRA.

Moreover, under the APA, EPA is due substantial deference for its regulatory determinations when the Agency is regulating in its area of special expertise at the very frontiers of science. This was explicated by the D.C. Circuit in *Natural Resources Defense Council v. v. Admin. U.S. EPA*.[260] We quote the Court's statement at length:

> In reviewing the primary standards for particulate matter, and the "adequacy" of the margin of safety, we are reviewing "predictions within an agency's area of special expertise, at the frontiers of science, " *New York v. EPA*, 852 F.2d 574, 580 (D.C. Cir. 1988), *cert. den.* 109 S.Ct. 1338 (1989). In such circumstances, we must defer to the agency's interpretation of equivocal evidence, so long as it is reasonable. *See id.* And where, as here, the statute is *"precautionary"* in nature, the evidence "uncertain or conflicting" and the "regulations designed to protect the public health," the court "will not demand rigorous step-by-step proof of cause and effect." *Ethyl Corp. v. EPA*, 541 F.2d 1, 28 (D.C. Cir.) (*en banc*), *cert. den.* 426 U.S. 941 (1976). "The Administrator may apply his expertise to draw conclusions from suspected, but not completely substantiated, relationships between facts, from trends among facts, from

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259 Achernar Broadcasting Co. v. F.C.C., 62 F.3d 1441, 1445 (D.C. Cir. 1995), quoting Kisser v. Cisneros, 14 F.3d 615, 619 (D.C. Cir. 1994).

260 902 F.2d 962 (D.C. Cir. 1990).

> theoretical projections from imperfect data, from probative preliminary data not yet certifiable as 'fact,' and the like." *Id.* But we must, nevertheless, carefully review the record to ascertain that the agency has made a reasonable decision based on "reasonable extrapolations from some reliable evidence." *Natural Resources Defense Council v. Thomas*, 805 F.2d 410, 432 D.C. Cir. 1986); *see also Marsh v. Oregon Natural Resources Council*, 109 S. Ct. 1851, 1861 (1989) (noting that courts must carefully review record and "satisfy[] themselves that the agency has made a reasoned decision based on its evaluation of the significance . . . of the . . . information.").[261]

EPA is confident that, should its regulatory decisions regarding the registration of *B.t.* plant-pesticides be subject to judicial review, they would fall well within the parameters set forth by the D.C. Circuit in *Natural Resources Defense Council*. In determining whether to register the *B.t.* plant-pesticides, and determining what terms and conditions are necessary for such registrations to prevent potential unreasonable adverse effects on the environment, EPA clearly is regulating "within [the] agency's area of special expertise, at the frontiers of science". Moreover, FIFRA is a <u>precautionary</u> statute that is intended to be implemented in a manner to protect the environment. Thus, EPA may apply its "special expertise" to address the uncertain data, evidence, and information currently available concerning the potential environmental impacts of *B.t.* plant-pesticides to make reasonable decisions based on "reasonable extrapolations from some reliable evidence." EPA believes that its registration decisions concerning *B.t.* plant-pesticides have constituted reasonable decisions based on reasonable extrapolations from reliable evidence. Further, EPA is committed to ensuring that any registration decisions regarding subsequent *B.t.* plant-pesticides will incorporate and address all available relevant data and information and will constitute reasonable decisions in the context of the APA.

2. <u>Petitioners' Specific Arguments Under the APA</u>

Petitioners' arguments in the "APA" section of the Petition merely reiterate scientific arguments made elsewhere and allege that the data and information presented demonstrate that EPA's registration actions and failure to cancel registrations are arbitrary, capricious, and an abuse of discretion. As discussed immediately above, EPA does not believe that Petitioners' arguments meet the APA standard for demonstrating that EPA engaged in arbitrary, capricious, and abusive decisionmaking in any respect. With respect to Petitioners' specific arguments, EPA cross references those sections of the Response that address the scientific issues reiterated by Petitioners and responds to novel arguments below.

(1) *With EPA on notice that B.t. plant-pesticides pose a risk of creating resistance, the registration of transgenic B.t. plant-pesticides under FIFRA without adequately*

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261 <u>Id.</u> at 968-69 (emphasis in original).

developing or assessing the adequacy of RMP is arbitrary, capricious, and an abuse of power. (27-28).

Petitioners' statement is conclusory and without basis. EPA believes that its ongoing actions regarding pest resistance management constitute adequate assessment of currently available information on pest resistance and its management. EPA discusses the current state of information regarding the risk that *B.t.* plant-pesticides will result in *B.t.* resistant pests above in section II.A.1.c.

(2) *Resistance management plans for transgenic B.t. plant-pesticides are based on two interacting premises requiring high toxin dose and presence of refugia. "Neither of these two premises has been scientifically validated in a manner which ensures the prevention of B.t. resistant pests."* (28)

EPA is not required to "ensure[] the prevention of *B.t.* resistant pests." (emphasis added). Moreover, it is doubtful that such a standard, if it existed, could be met. Under FIFRA, in order to grant a registration for the sale and distribution of a pesticide, EPA must determine that the pesticide will not cause "unreasonable adverse effects" to human health or the environment. EPA has undertaken a comprehensive and ongoing program of review and consultation with experts to determine what steps are necessary and scientifically justified to prevent development of *B.t.* resistant pests. On the basis of this ongoing review and consultation, EPA has developed a multi-step PRM plan. This plan includes more than just high dose expression + refugia, notwithstanding that these are very important components of PRM strategy. EPA discusses the current state of information regarding the risk of *B.t.* plant-pesticides resulting in resistant pests above in section II.A.1.c.[262]

(3) *EPA's RMP strategy fails to address several critical issues including occurrence of multiple pest species and enforcement protocols ensuring compliance with RMPs.*

EPA acknowledges that aspects of current Pesticide Resistance Management (PRM) plans may be capable of improvement. To continue its ongoing policy of regulating *B.t.* plant-pesticides in accordance with the latest available scientific understanding, EPA plans to undertake, in the year 2000, a comprehensive reassessment, including pest resistance management, for *B.t.* corn and cotton. This comprehensive reassessment will include (1) consideration by EPA of all currently available information

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262 Petitioners' assertions that (1) *a known instance of limited efficacy of B.t. cotton demonstrates that the high dose toxin production requirement "has not been adequately understood or assessed"* (Petition at 28); (2) *new research suggests that pests have the ability to discriminate among tissues with varying concentrations of toxin within a plant* (Petition at 28); and (3) *there exists no scientific consensus on the temporal or geographic arrangement or the size of effective refuges* (Petition at 28-29), are addressed above in section II.A.1.c.,d., and e.

on the risks and benefits of *B.t.* corn, cotton, and potato plant-pesticides; (2) development by EPA scientists of an updated risk assessment for *B.t.* plant-pesticides; (3) outside scientific peer review of EPA's risk assessment by the FIFRA Scientific Advisory Panel (SAP); (4) public comment on EPA's risk assessment; and (5) other opportunities for public involvement. This comprehensive reassessment will enable EPA to determine the appropriate terms and conditions of any subsequent *B.t.* plant-pesticide registrations to ensure adequate IRM. Also, based upon experience with the current *B.t.* plant-pesticide registrations, EPA believes that one area that requires improvement in any subsequent registrations is enforcement mechanisms for ensuring that all PRM information reporting requirements are met on a timely basis, and that there is compliance by substantially 100% of growers and registrants with all PRM requirements.

E. Public Trust Doctrine

There is no basis for Petitioners' attempt to expand federal common law to effect application of the public trust doctrine to EPA's regulatory activities regarding pesticide registrations under FIFRA. The D.C. Circuit has noted that the question of whether the public trust doctrine even applies to the federal government has not been resolved by the courts.[263] The Supreme Court has only applied the public trust doctrine to states.[264] Furthermore, traditionally, the public trust doctrine was applied solely to water resources.[265] Petitioners' argument that there is a public trust duty at issue here is premised on their conclusion that the federal government's responsibilities with respect to the genetic material of insects is the same as its responsibility, if indeed it has any, for navigable waters.[266] Because there is no federal common law which has yet developed addressing federal responsibility for genetic material, Petitioners are essentially asking the Court to create new substantive federal common law. Given the

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263 District of Columbia v. Air Florida, Inc., 750 F.2d 1077, 1083-84 (1984). The Court of Appeals noted that the issue had only been considered by a few federal district courts. 750 F.2d at 1083-84 & n.37. Subsequently, two other district courts have concluded that the public trust doctrine does not apply to the federal government. U.S. v. 11.037 Acres of Land, 685 F. Supp. 214, 217 (N. D. Cal. 1988) (holding that United States' condemnation of California land extinguishes public trust easement); Sierra Club v. Block, 622 F. Supp. 842 (D. Colo. 1985) (dismissing public trust doctrine claim under Fed. R. Civ. P. 12(b)(6)).

264 See, e.g., Phillips Petroleum Co. v. Mississippi, 484 U.S. 469, 473 (1988) (Mississippi); Illinois Central Railroad v. Illinois, 146 U.S. 387, 435 (1892); (Illinois); Shively v. Bowlby, 152 U.S. 1, 14-15 (1894) (Oregon).

265 See Phillips Petroleum Co., 484 U.S. at 476; Air Florida, 750 F.2d at 1082.

266 Petition at 30-31.

very limited nature of the courts' authority to develop federal common law, however,[267] there is no legal basis for Petitioners' arguments that EPA can base actions concerning FIFRA registrations on that doctrine. Such questions regarding national policy on new and complicated issues should be addressed by Congress in the first instance, certainly not by an administrative agency.[268]

CONCLUSION

For the reasons set forth above, EPA denies Petitioners' requests and determines that granting the relief requested by Petitioners is not required pursuant to the Agency's obligations under FIFRA, NEPA, the APA, or any other applicable Federal statutes.

Sincerely Yours

Susan Wayland
Assistant Administrator

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267 Texas Indus., Inc. v. Radcliff Materials, Inc., 451 U.S. 630, 641 (1980).

268 Cf. City of Milwaukee, 451 U.S. at 314. The other line of non-water related cases cited by petitioners, Petition at 30 n.101, regarding a "public trust" are not applicable because those cases were concerned with the disposition and/or sale of federally-owned lands. Moreover, under that line of cases, the Supreme Court has held that Congress has the sole discretion as to how to utilize such lands and resources. Alabama v. Texas, 347 U.S. 272, 277 (1954); Kleppe v. New Mexico, 426 U.S. 529, 539 (1976)(citing United States v. San Francisco, 310 U.S. 16, 29 (1940)); Light v. United States, 220 U.S. 523, 537 (1911).

APPENDIX A

Pesticide Regulation Pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

Pesticide Regulation

Under FIFRA, EPA has the authority to regulate the development, sale, distribution, use, storage, and disposal of pesticides.[269] EPA's primary mission under FIFRA is to ensure that pesticides do not pose unreasonable adverse effects on human health or the environment. The statute makes it unlawful, with a few minor exceptions, for any "person in any State [to] distribute or sell to any person any pesticide that is not registered" under the Act.[270] Thus, a registration granted by EPA under FIFRA is a license that establishes the terms and conditions under which the pesticide may be lawfully sold and used.[271]

1. Registration Requirements and Procedures

Section 3 of FIFRA sets forth the procedures and standards for registering a pesticide. Applicants must file a statement containing the information identified in section 3(c)(1) and must submit data supporting the registration as required by section 3(c)(2).[272] Under section 3, EPA must register a pesticide if the Agency determines that the pesticide meets certain enumerated criteria. The burden of proving that a pesticide satisfies the standard is on the proponent of the registration, and continues as long as the registration remains in effect.[273]

Under Section 3(c)(5), EPA must register a pesticide if the Agency determines that (1) the pesticide's composition warrants claims that are to be made with it, (2) the materials, including the labeling, comply with FIFRA's requirements, (3) the use of the pesticide will not cause "unreasonable adverse effects on the environment," and (4) when used "in accordance with widespread and commonly recognized practice" it will not cause unreasonable adverse effects on the environment.[274]

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269 In conjunction with EPA's pesticide registration responsibilities under FIFRA, Section 408 of the Federal Food, Drug, and Cosmetic Act ("FFDCA"), 21 U.S.C. §§ 321 et seq., requires the establishment of maximum legally permissible levels (tolerances), or an exemption from the requirement of a tolerance, for any pesticide residues used in connection with food or animal feed. 21 U.S.C. § 346a.

270 7 U.S.C. § 136a(a); see also 7 U.S.C. § 136j(a)(1).

271 See 7 U.S.C. §§ 136a(c)(1)(A)-(F), 136a(d)(1).

272 7 U.S.C. § 136a(c)(1)-(2).

273 Industrial Union Dep't v. American Petroleum Inst., 448 U.S. 607, 653 n.61 (1980).

274 7 U.S.C. § 136a(c)(5).

FIFRA section 2(bb) defines "unreasonable adverse effects on the environment" to mean: "(1) any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide, or (2) a human dietary risk from residues that result from a use of a pesticide in or on any food inconsistent with the standard under section 408 of the Federal Food, Drug, and Cosmetic Act."[275] In order to demonstrate that its pesticide product meets the standard for registration, including a demonstration that the pesticide will not cause unreasonable adverse effects on the environment, an applicant must submit sufficient data to the Agency demonstrating that the product meets this standard.[276] EPA has promulgated regulations detailing the data requirements needed for registration. These requirements are located at 40 C.F.R. Part 158. Part 158 contains requirements for basic product chemistry data, human health and safety data, environmental fate and effects data as well as pesticide residue data.[277]

FIFRA also authorizes EPA, under specified circumstances, to issue a "conditional" registration under section 3(c)(7).[278] Section 3(c)(7)(A) authorizes a conditional registration or amendment to an existing registration of a pesticide that is identical or substantially similar to any currently registered pesticide, or that differs only in insignificant ways, and that would not significantly increase the risk of unreasonable adverse effects. Applicants for conditional registration under Section 3(c)(7)(A) must submit all data as are required for registration under Section 3(c)(5).[279] Section 3(c)(7)(B) authorizes a conditional amendment to a registration to permit additional uses of an already-registered pesticide provided the additional use would not significantly increase the risk of any unreasonable adverse effect on the environment. Applicants for conditional registration under Section 3(c)(7)(B) must submit all

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275 7 U.S.C. §136(bb). In 1995, however, at the time of registration of four of the subject transgenic *B.t.* endotoxins (Cry3A in potato, Cry1Ac in cotton, and Cry1Ab in field corn), the FIFRA definition of "unreasonable adverse effects on the environment" only contained the first criterion of unreasonable risk to man or the environment. Congress subsequently expanded the definition of "unreasonable adverse effects on the environment" in 1996, adding the second criterion regarding consistency with the FFDCA section 408 standard.

276 Id. § 136a(c)(1)(F).

277 Part 158 contains a standard set of data requirements; however, certain data may be waived by the Agency on a case-by-case basis in response to a request by the applicant. 40 C.F.R. § 158.45. The Agency may also impose additional data requirements for certain products if the data routinely required is insufficient to permit an evaluation of the potential of the product to cause unreasonable adverse effects on the environment. Id. § 158.65.

278 7 U.S.C. § 136a(c)(7).

279 Id. §136a(c)(7)(A).

data as are required for registration under Section 3(c)(5).[280] Section 3(c)(7)(C) authorizes a conditional registration for a pesticide that contains a new active ingredient not present in a currently registered pesticide when the Agency has imposed a data requirement and "a period reasonably sufficient for generation of the data has not elapsed." EPA may issue this type of conditional registration for a period of time sufficient for the generation and submission of the required data if EPA determines that "use of the pesticide during such a period will not cause any unreasonable adverse effect on the environment, and that use of the pesticide is in the public interest."[281]

If the Agency determines that the application cannot meet the criteria under either 3(c)(5) or 3(c)(7), the Agency may deny the application.[282] Upon such notification by the Agency, the applicant is authorized to pursue the remedies provided under section 6 of the Act, which include the right to request a hearing before the Agency.[283] This hearing is the same in all relevant respects as the hearing to which a registrant is entitled when the Agency proposes to cancel its registration.

2. Cancellation

Section 6(b) of FIFRA provides for the cancellation of pesticide registrations.[284] If it appears to EPA that a pesticide does not comply with FIFRA or, when used in accord with widespread practices "generally causes unreasonable adverse effects on the environment," EPA may issue a notice of its intent to either (1) cancel the registration or change its classification or (2) hold a hearing to determine whether the registration should be canceled.[285] Before issuing such a notice, EPA must coordinate with the Secretary of the Department of Agriculture. EPA must then issue the notice to the registrant and to the public. If EPA issues a notice of intent to cancel the registration, rather than a notice of intent to hold a hearing, cancellation becomes final 30 days after (1) the registrant has received notice <u>and</u> (2) the notice has been published, unless the registrant corrects the defects or "a person

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[280] <u>Id.</u> §136a(c)(7)(B).

[281] <u>Id.</u> §136a(c)(7)(C).

[282] 7 U.S.C. § 136a(c)(6); 40 C.F.R. § 152.118(a).

[283] 7 U.S.C. § 136a(c)(6); <u>see also</u> 7 U.S.C. § 136d; 40 C.F.R. Part 164.

[284] 7 U.S.C. § 136d(b).

[285] <u>Id.</u>

adversely affected by the notice" requests a hearing.[286] If a hearing is requested, the final decision on cancellation will be issued after completion of the hearing in accord with EPA's regulations.[287]

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286 Id.

287 40 C.F.R. Part 164.

APPENDIX B

Scientific and Technical Public Meetings Held on *B.t.* Plant-Pesticide Science and Policy Issues

EPA has conducted a number of public meetings regarding the science and policy issues for plant-pesticides in general and *B.t.* plant-pesticides in particular. These were held both prior and subsequent to the proposal of the plant-pesticide rules. The Agency has held six EPA Biotechnology Science Advisory Committee or Office of Pesticide Program (OPP) FIFRA Scientific Advisory Panel (SAP) Meetings,[288] two Pesticide Program Dialogue Committee (PPDC) meetings,[289] two EPA public hearings, and three stakeholder workshops. The Agency has also sponsored, or cosponsored with other Federal agencies, four conferences dealing with plant-pesticides and the pertinent data needed to perform a risk assessment. These meetings are described below. The documents and reports of these meetings are available in the public docket for this Response.

EPA Biotechnology Science Advisory Committee/FIFRA SAP meetings

On six occasions, the Agency has requested the advice of various scientific advisory panel committees on FIFRA and FFDCA-related scientific issues for the regulation of plant-pesticides. On December 18, 1992, a subpanel of the FIFRA Science Advisory Panel (SAP) was convened to review a draft policy statement on plant-pesticides and respond to a series of scientific questions posed by EPA's approach under FIFRA. On July 13, 1993, a Subcommittee of the EPA Biotechnology Science Advisory Committee (BSAC) was convened to address a series of scientific questions primarily on EPA's approach under FFDCA. On January 21, 1994, a joint meeting of the SAP/BSAC subpanel on plant-pesticides was held to discuss additional scientific questions. On March 1, 1995, a subpanel of the FIFRA SAP was convened to review the Agency's risk assessment and resistance management analysis for *B.t.* potato (Cry3A). On February 9-10, 1998, a subpanel of the FIFRA SAP met to discuss insect resistance management issues for *B.t.* crops. Finally, on December 8, 1999, a subpanel of the FIFRA SAP met to discuss the current product characterization, environmental fate, and non-target organism data requirements for protein plant-pesticides.

The December 19, 1992 subpanel reviewed the Agency's draft policy statement on plant-pesticides and responded to a series of scientific questions posed by the Agency primarily on EPA's approach under FIFRA. On July 13, 1993, a Subcommittee of the EPA Biotechnology Science Advisory Committee (BSAC) was convened to address a series of scientific questions primarily on EPA's approach under FFDCA. On January 21, 1994, a joint meeting of the SAP/BSAC subpanel on plant-pesticides was held. The subpanel noted a number of questions regarding the Agency's proposed policy under FIFRA and FFDCA and guidance for data needs for the evaluation of plant-pesticides. The December 1992 subpanel also addressed the issue of development of pest resistance

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[288] The OPP FIFRA SAP is composed of non-Agency scientists who perform peer reviews of the Agency's scientific risk assessments and guidelines for pesticide regulation.

[289] The OPP Pesticide Program Dialogue Committee (PPDC) is composed of representatives of different stakeholder organizations who provide the Agency input on new and ongoing policy developments related to pesticide regulation.

to a pesticidal substance produced by plants. The subpanel stated that delaying the evolution of resistance was very important and urged EPA to actively assess the problem of pesticide resistance, especially when the pesticide is part of the progression toward use of "safer" pesticides. A summary of the issues raised at these meetings and the Agency's response are discussed in EPA's draft policy document for plant-pesticides.[290]

The March 1, 1995 SAP Subpanel met to discuss the human health and environmental risk issues primarily associated with *B.t.* potato, the first *B.t.* plant-pesticide under consideration by the Agency for a full registration. The SAP subpanel indicated that the Agency adequately addressed the human health and environmental risk issues. The SAP subpanel indicated that Naturemark (Monsanto) presented a scientifically credible Colorado potato beetle (CPB) resistance management protocol for their Newleaf potato variety. In addition, the SAP Subpanel stated that "the short-term and long-term strategies proposed by Monsanto to delay CPB resistance are appropriate and as long as Monsanto remains flexible and quickly responds to new data, their long-term strategies appear to be appropriate."[291]

The February 9-10, 1998 FIFRA SAP subpanel specifically examined the resistance management strategies for *B.t.* delta-endotoxins expressed in potatoes, field corn, and cotton. Prior to this meeting of the FIFRA SAP, EPA wrote a white paper entitled *The Environmental Protection Agency's White Paper on Bacillus thuringiensis Plant-Pesticide Resistance Management* [EPA 739-S-98-001] (EPA, 1998). This paper described EPA's scientific assessment of the current resistance management plans for *B.t.* plant-pesticides for *B.t.* potato, *B.t.* corn and *B.t.* cotton following the first year (1996) of full-scale commercial release and also discussed possible future requirements for the successful implementation of long-term (sustainable) resistance management for *B.t.* plant-pesticides. The white paper also included a summary of the two public hearings hosted by the Agency in 1997. EPA asked the SAP subpanel to review specific questions based on the "White Paper." The

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290 Plant-pesticides subject to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA), 59 Fed. Reg. 60496-60547 (proposed policy) (November 23, 1994).

291 Scientific Advisory Panel, Subpanel on Plant-Pesticides, p.4 (March 1, 1995) (Docket Number: OPP-00401). The Subpanel considered a set of scientific issues in connection with Monsanto Company's application for registration of a plant-pesticide containing the active ingredient *Bacillus thuringiensis* subsp. *tenebrionis* delta-endotoxins. Although the 1995 SAP meeting focused primarily on the review of the risk assessment and resistance management issues for *B.t.* potatoes, the FIFRA SAP subpanel also generally discussed resistance management issues for potential *B.t.* corn and *B.t.* cotton plant pesticide registrations.

subpanel provided the Agency with a final report of the meeting on April 28, 1998.[292] In connection with this SAP subpanel meeting, the Agency received oral and written statements from approximately 20 different groups representing industry, growers or grower groups, trade organizations, academia, and environmental groups.

The December 8, 1999 FIFRA SAP subpanel discussed current data requirements related to the product characterization, environmental fate, and non-target organism for protein plant-pesticides. EPA presented the current thinking for data requirements regarding product characterization. For protein plant-pesticides, characterization includes not only the genetic constructs, vectors, and proteins produced, but also information on the biology of the host plant, expression of the protein, and the potential for genetic transfer. The Agency also presented its current thinking for data requirements regarding environmental fate and non-target effects. The SAP released its report February 4, 2000.[293] The panel commended and generally supported EPA's current approach. The panel supported EPA's current approach to the product characterization data, the need for additional field monitoring regarding impacts to non-target insects, and testing methodology for non-target insects. The panel also provided specific comments regarding program improvements, including the need for additional studies. The specific recommendations in the report are measures that can be included in our current program, and are consistent with many of the scientific enhancements the Agency is already implementing.

ii. PPDC meetings

The Office of Pesticide Program's Pesticide Program Dialogue Committee (PPDC) is composed of representatives of different stakeholder organizations who provide the Agency input on new and ongoing policy developments related to pesticide regulations. The minutes of the PPDC are available at http://www.epa.gov/pesticides/ppdc. The Agency has raised, in general, the issue of pesticide resistance management to its Pesticide Program Dialogue Committee (PPDC) in July 1996 and, most recently, in January 1999. At the July 1996 meeting, the PPDC indicated that EPA should play a role in pest resistance management and should not make resistance management mandatory in all cases. However, the PPDC indicated that EPA should act in the "public good" to protect the efficacy of certain pesticide products that are safer or provide unique public health or other societal benefits.

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292 Scientific Advisory Panel, Subpanel on *Bacillus thuringiensis* (Bt) Plant-Pesticides, February 9- 10, 1998, Transmittal of the final report of the FIFRA Scientific Advisory Panel Subpanel on Bacillus thuringiensis (Bt) Plant-Pesticides and Resistance Management, Meeting held on February 9-10, 1998 (1998) (Docket Number: OPPTS-00231).

293 Scientific Advisory Panel, Subpanel on *Bacillus thuringiensis* (Bt) Plant-Pesticides, December 8, 1999, Characterization and Non-Target Organism Data Requirements for Protein Plant-Pesticides. The report is available to the public on the SAP web site at www.epa.gov/scipoly/sap and in the docket for this action.

The PPDC agreed that genes from *Bacillus thuringiensis* (*B.t.*) were a special case ("in the public good") and worthy of extra protection.[294]

At the January 1999 meeting, EPA provided an update of insect resistance management for *B.t.* crops, USDA introduced its proposal for setting up virtual regional pest management centers, and an Industry Coalition (Pioneer, Monsanto/Dekalb, Mycogen/Dow, and Novartis) introduced a new IRM proposal for a single refuge of 20% (sprayable) for *B.t.* corn in the Corn Belt.[295] The PPDC continued to support EPA's actions to protect *B.t.* and encouraged EPA to provide more information regarding an explicit compliance/enforcement plan for resistance management for the general public.[296] USDA presented the concept of having "virtual" regional pest management centers based on similarity of cropping patterns, pest problems and environmental conditions. The regional centers would provide a forum for a discussion of pest management issues for pesticides as part of the implementation of the more stringent food safety standards mandated by the FQPA. These regional centers could also assist in the development and establishment of appropriate regional resistance management strategies for *B.t.* crops.

iii. Public hearings

EPA also held two public hearings, one on March 21, 1997 (Washington D. C.) and the other on May 21, 1997 (College Station, Texas), to solicit comments on insect resistance management plans for *B.t.* plant-pesticides. There were four issues open for comment in these meetings: (1) the requirement for resistance management plans, (2) scientific needs for resistance management plans, (3) use of "public good" as a criterion for the requirement of resistance management plans, and (4) performance of *B.t.* cotton. One of the reasons for these two public hearings was the 1996 reports that *B.t.* cotton failures were related to cotton bollworm resistance, especially in Texas. As part of Monsanto's registration agreement for *B.t.* cotton, Monsanto reported these incidents of purported resistance to EPA, analyzed these incidents, and implemented responsive action. EPA evaluated the information/data generated by the Monsanto Company on the incidents involving the company's *B.t.* cotton and the cotton bollworm in 1996. All evidence available indicated that there was no resistance by cotton bollworm to the Cry1Ac delta-endotoxin produced in *B.t.* cotton. Approximately 100

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294 Pesticide Program Dialogue Committee. Pesticide Program Meeting Summary. July 9-10, 1996. http://www.epa.gov/pesticides/ppdc.

295 Pesticide Program Dialogue Committee. Transcript of Meeting of the Pesticide Program Dialogue Committee January 7 - 8, 1999. http://www.epa.gov/pesticides/ppdc .

296 Id.

individuals/organizations submitted written comments and/or delivered presentations regarding the subject of *B.t.* plant-pesticide resistance management and the four issues open for comment.[297]

iv. EPA-sponsored conferences

EPA has sponsored, or cosponsored with other Federal agencies, four conferences dealing with plant-pesticides and the pertinent data needed to perform a risk assessment: (1) a meeting on "Genetically Engineered Plants: Regulatory Considerations" at Cornell University, Ithaca, New York, October 19-21, 1987[298]; (2) a "Transgenic Plant Conference" at Annapolis, Maryland, September 7-9, 1988[299];(3) a "Conference on Pesticidal Transgenic Plants" at Annapolis, Maryland, November 6-7, 1990[300], and (4) a "Conference on Scientific Issues Related to Potential Allergenicity in Transgenic Food Crops" at Annapolis, Maryland, April 18-19, 1994[301].

v. Workshops

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297 These hearings are discussed in the Agency's "White Paper" on *B.t.* Plant-Pesticide Resistance Management. Copies of the written comments are available in the Office of Pesticide Programs public docket, OPP-00470.

298 Boyce Thompson Institute. 1987. Regulatory considerations: genetically engineered plants. San Francisco: Center for Science Information.

299 U.S. Environmental Protection Agency, U.S. Food and Drug Administration, and U.S. Department of Agriculture. 1988. Proceedings - Transgenic Plant Conference, September 7-9, 1988, Annapolis, Maryland.

300 U.S. Environmental Protection Agency. 1990. Pesticidal Transgenic Plants: Product Development, Risk Assessment, and Data Needs. U.S. EPA Conference Proceedings, November 6 and 7, 1990. EPA/21T-1024.

301 U.S. EPA, U.S. Food and Drug Administration, and USDA. Transcript of "Conference on Scientific Issues Related to Potential Allergenicity in Transgenic Food Crops" at Annapolis, Maryland, April 18-19, 1994. FDA Doc. 94N-0053, document TR-1. A summary of the conference is found at http://vm.cfsan.fda.gov/~lrd/bioallrg.html .

EPA held a workshop on plant-pesticides in Washington, D.C. on July 17-18, 1997.[302] EPA provided stakeholders with an overview of its proposed plant-pesticide regulations and held panel discussions on the proposed regulations and their potential impact on different stakeholders.

EPA and USDA held two one-day public workshops on insect resistance management for Bt crops in 1999.[303] The first meeting was held in Chicago on June 18, 1999 and focused on *B.t.* corn. The second meeting was held in Memphis on August 26, 1999and focused on *B.t.* cotton. The meetings provided all stakeholders an opportunity to better understand the issues regarding insect resistance management in Bt crops. EPA and USDA jointly authored a position paper used at this workshop entitled "EPA and USDA Position Paper on Insect Resistance Management (IRM) in Bt Crops." This paper sets forth the EPA-USDA positions on Bt potato, Bt corn, and Bt cotton and was used by stakeholders as a basis of discussion at the workshop. Stakeholders at the workshops discussed the following issues: Refuge Design and Deployment, Education and Compliance, and Monitoring and Remedial Action. These workshops explored ways of designing and implementing sustainable resistance management programs that are flexible and can accommodate rapidly changing technology (e.g., stacking genes, novel *B.t.* genes, or other novel genes). They provided a forum for discussion and consensus-building on what should be implemented as sustainable insect resistance management (IRM) programs for the year 2000 and future years.

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302 A. Ann Sorenson (Ed.) 1997. Environmental Protection Agency Plant-Pesticide Workshop. Washington, D.C. July 17-18, 1997. National Foundation for Integrated Pest Management Education.

303 All materials for the *B.t.* Transgenic Crop IRM workshops, including the EPA-USDA position paper and summaries of the meetings, are located at http://www.epa.gov/pesticides/biopesticides/news_archive.htm .

APPENDIX C

List of Registered *B.t.* Plant-pesticides

Registered B.t. plant-pesticides

There are currently ten separate registered products containing *B.t.* endotoxins in potato, corn (field corn, popcorn, and sweet corn), and cotton shown in Table 1 below.

Table 1. Registered *B.t.* Plant-Pesticides for Commercial Use

EPA Registration Number	Date Registered	Events[304]/ Products	Toxin	Crop	Company(s)
524-474	May 1995	NewLeaf®	Cry3A	Potato	Monsanto
524-498	Nov 1998	NewLeaf Plus®	Cry3A and potato leaf roll virus replicase	Potato	Monsanto
66736-1	Aug 1995	176	Cry1Ab	Field Corn	Novartis Seeds
66736-1	Mar 1998	176	Cry1Ab	Popcorn	Novartis Seeds, Inc.- Field Crops-NAFTA
68467-1	Aug 1995	176	Cry1Ab	Field Corn	Mycogen Corp.
67979-1	Aug 1996	BT11	Cry1Ab	Field Corn	Novartis Seeds
65269-1	Feb 1998	BT11	Cry1Ab	Sweet Corn	Novartis Seeds, Inc. - Vegetables - NAFTA
524-489	Aug 1996	MON810	Cry1Ab	Field Corn	Monsanto
69575-2	Apr 1997	DBT-418	Cry 1Ac	Field Corn	DeKalb Genetics Corp.
45639-221	May 1998	CBH-351	Cry9C	Field Corn	AgrEvo
524-478	Oct 1995	Bollgard®	Cry1Ac	Cotton	Monsanto

304 Event denotes the successful transformation of a crop plant by specific insertion of the genetic material into a specific crop plant that lead to the commercialized *B.t.* crop hybrid.

APPENDIX D

Acronyms and Definitions

Colorado potato beetle (CPB) - *Leptinotarsa decemlineata* (Say)

Cotton bollworm (CBW) (also known as corn earworm - CEW) - *Helicoverpa zea* (Boddie)

Cross-resistance - Resistance to one toxin that conditions resistance to another.

Diagnostic dose - a dose of toxin that enables diagnosis of the susceptibility of the insect tested in a bioassay with reasonable accuracy. Unlike a discriminating dose, a diagnostic dose may allow survival of some susceptible individuals and kill some resistant individuals. If responses of susceptible and resistant individuals overlap, it is possible to find a diagnostic dose, but not a discriminating dose.

Discriminating dose - A dose of toxin that can distinguish between resistant and susceptible individuals. Unlike the diagnostic dose, the discriminating dose kills all susceptible individuals but no resistant individuals and thus can be used in a bioassay to discriminate between the two types of individuals. In some cases, a discriminating dose will also distinguish between heterozygote and homozygote individuals.

European corn borer (ECB) - *Ostrinia nubilalis* (Hübner)

Event - Event denotes the successful transformation of a crop plant by specific insertion of the genetic material into a specific crop plant that lead to the commercialized *B.t.* crop hybrid. For *B.t.* field corn: Event 176 hybrids express Cry1Ab (Novartis and Mycorgen), BT11 hybrids express Cry1Ab (Novartis), DBT-418 hybrids express Cry1Ac (DeKalb), MON810 hybrids express Cry1Ab (Monsanto), CBH-351 hybrids express Cry9C (AgrEvo/Aventis).

Fall armyworm (FAW) - *Spodoptera frugiperda* (J.E. Smith)

High dose - A dose of toxin high enough to kill all or nearly all susceptible individuals. Such a dose is theoretically possible by plants with sufficiently high concentration of *B.t.* toxin. In practice, however, all heterozygous genotypes cannot be identified and thus a precise concentration level cannot be specified. An operational definition has been provided by the 1998 SAP, who defined a high dose as twenty-five times the amount of B.t. delta-endotoxin necessary to kill susceptible insects.

High dose/refuge strategy - An insect resistance management approach for minimizing the rapid selection for resistance to transgenic plants. This strategy uses plants that produce Cry proteins at a concentration sufficient to kill all but the most resistant insects in combination with non-Bt plants that allows susceptible insects to survive and randomly mate with resistant individuals to dilute resistance.

Insect resistance management (IRM) - A proactive process of limiting or delaying insect resistance development in a pest population with a focus on preserving susceptible genes (individuals).

Integrated pest management (IPM) - A management approach that integrates multiple, complementary control tactics (e.g., biological control, crop rotation, host plant resistance , and pesticides) to manage pests.

LC_{50} - The concentration of Bt toxin in a plant that kills 50% of the individuals being tested.

LC_{99} - The concentration of Bt toxin in a plant that kills 99% of the individuals being tested.

Phenology - The periodicity or timing of events within an organism's life cycle, e.g., flowering time.

Pink bollworm (PBW) - *Pectinophora gossypiella* (Saunders)

Polyphagous - term describing insects that feed on multiple hosts, e.g., *Helicoverpa zea* (Boddie)

Refuge - Areas that are untreated with a particular pesticide in order to leave portions of the pest population unexposed to that insecticide. For *B.t.* crops, a refuge is a stand of non-*B.t.* host plants that are managed to provide sufficient susceptible adult insects to mate with potential *B.t.*-resistant adult insects to dilute the frequency of resistance genes.

Resistance - The evolved capacity of an organism to survive in response to a selective pressure from exposure to a pesticide. The evolution of resistance occurs through a process of genetic accumulation (i.e., selection) whereby a population becomes less sensitive to the pesticide. The point where an organism is declared resistant is frequently defined by an arbitrarily defined level in computer simulation models or operationally in pest management as when a product is judged to provide adequate protection from the pest in the field.

Southwestern corn borer (SWCB) - *Diatraea grandiosella* (Dyar)

Tobacco budworm (TBW) - *Heliothis virescens* (Fabricius)