006463

(9) A or

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

22pp

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MAR 24 1997

PUBLIC DOCKET

MEMORANDUM

SUBJECT: Consideration of Section 3(c)(7)(C) Conditional Registration for Dekalb Genetic Corporation's *Bt* Corn Plant-pesticide: *Bacillus thuringiensis* subspecies *kurstaki* CryIA(c) Delta-Endotoxin and the Genetic Material Necessary for It's Production in Corn (EPA File Symbol 69575-E)

-DECISION MEMORANDUM-

FROM: Janet L. Andersen, Director
Biopesticides and Pollution Prevention Division

TO: Daniel M. Barolo, Director
Office of Pesticide Programs

I. ISSUE

Should the Agency conditionally register Dekalb's Bt corn product for full commercial use in field corn pursuant to FIFRA §3(c)(7)(C)?

II. SUMMARY AND REGULATORY BACKGROUND

A. Experimental Use Permit and Registration Applications

On 5/2/96, the Agency issued a one year crop destruct experimental use permit for Dekalb's *Bt* corn, EPA EUP No. 69575-EUP-1.

On 11/30/95 Dekalb submitted an application for a seed increase registration for their Bt corn, EPA File Symbol 69575-R.

On 4/23/96 Dekalb submitted an application for a full commercial use registration for their Bt corn, EPA File Symbol 69575-E.

1922

B. Tolerance Petitions

On 4/23/96 Dekalb applied for exemptions from the requirement of a tolerance for *Bacillus thuringiensis* subpsecies *kurstaki* CryIA(c) protein and the genetic material necessary for its production in all plant raw agricultural commodities (PP 4F4711) and Phosphinothricin acetyltransferase (PAT) and the genetic material necessary for its production in all plant raw agricultural commodities (PP 4E4710).

C. Plant-Pesticide Policy

The Agency published its proposed position on the regulation of pesticidal substances produced in plants (**59 FR 60496, November 23, 1994**). In the proposal, the Agency would designate the pesticidal substances produced by plants as plant-pesticides. In addition, the Agency issued proposed regulations that define certain categories of plant-pesticides that would be exempt from regulation under FIFRA and FFDCA. Plant-pesticides not exempt would be subject to regulation. The *Bacillus thuringiensis* delta-endotoxins are examples of plant-pesticides that would continue to be regulated under the proposal.

D. Conclusion

The Biopesticides and Pollution Prevention Division (BPPD) has evaluated the data submitted and/or cited by Dekalb. Based on these data and other relevant information, OPP believes that outstanding data in the area of insect resistance management exist. However, insufficient time has elapsed to develop these data since the Agency made a final determination that they would be required. BPPD recommends granting a conditional registration, because the registration is in the public interest and otherwise meets the requirements of FIFRA section 3(c)(7)(C).

BPPD scientists have reviewed the information submitted with respect to health effects, and these data show that the product will be digested like any other protein and genetic material and will have no significant effects on human health. Likewise, the data submitted for ecological effects have identified no significant hazards to non-target organisms. The pesticide resistance management plan submitted by Dekalb has been reviewed. Certain terms and conditions were deemed necessary for these registrations in order to mitigate the risk of insect resistance to *Bacillus thuringiensis*.

The registration for use of this product in field corn, if granted, would be conditional under section 3(c)(7)(C) of FIFRA. The Agency is imposing terms and conditions as outlined in Section V of this document to address resistance management concerns. These conditions and terms are being imposed to address concerns that European corn borer (ECB) and corn earworm (CEW) may develop resistance to *Bt* plants and sprays which can be used on corn, cotton, and a variety of vegetable crops.

2722

III. PUBLIC COMMENTS TO NOTICE OF RECEIPT

A. Comment in Response to Seed Increase and Full Commercial Use of this Plant-Pesticide

The Agency received no comments in response to the Dekalb applications and petitions mentioned above.

B. General Comments in Response to *Bt* Corn

Several comments were received and addressed in the Ciba/Mycogen *Bt* corn decision document of August, 1995. The main concern expressed by commentors was that of insect resistance development. In addition, over 800 comments calling for the Agency to have "workable resistance management plans" before allowing further *Bt* plant-pesticide registrations have been received by the Agency. The Agency believes that the terms and limitations of this registration relative to resistance management adequately mitigate the risk of resistance developing during the duration of this conditional registration.

Margaret Mellon and Jane Rissler of the Union of Concerned Scientists and Rebecca Goldberg of the Environmental Defense Fund sent a letter dated 10/10/96 to Assistant Administrator for Prevention, Pesticides, and Toxic Substances, Dr. Lynn R. Goldman. The letter stated that Bt resistance management is at a critical stage and they requested that EPA: (1) convene a meeting of the FIFRA Scientific Advisory Panel (SAP) in the Fall of this year to evaluate current Bt resistance management efforts, (2) prepare a report on the status of resistance management, and (3) suspend registrations of Bt-corn and Bt-cotton and delay approvals of other Bt crops.

The request that the EPA should convene a meeting within the FIFRA SAP to evaluate resistance management efforts has merit. However, SAP meetings are planned several months in advance. The Office of Prevention, Pesticides, and Toxic Substances (OPPTS) is exploring having either a SAP meeting or similar forum in 1997. In order to have a productive meeting several preparatory steps must occur before such a meeting is convened.

First, the EPA has to evaluate the information/data being generated by the Monsanto Company on the incidents involving the company's Bt-cotton and the cotton bollworm in 1996. Monsanto has submitted their information to EPA and the Agency has begun the evaluation process. Before EPA can complete this review, however, certain pieces of information outlined in the next paragraph must be available for comparative analysis. EPA's review of these data along with any data that are not considered confidential business information (CBI), should be made available at a SAP meeting. A cursory review of the submitted information indicates that Monsanto conducted a number of assays and studies to investigate the lack of effective cotton bollworm control in Texas. The Monsanto submission does not provide all of the details necessary for a final review on the validity of Monsanto's assertion that bollworm failures were due largely in response to record cotton bollworm population densities in 1996.

Monsanto has been contacted regarding this deficiency and is preparing a supplemental submission to address these Agency concerns.

Second, EPA must have an opportunity to evaluate the use pattern data and research data being supplied by the registrants to EPA for Bt corn and Bt-cotton. Annual information reports are due to EPA each January 31st, and have been submitted. EPA believes these reports will serve as the basis for any presentation at such a public meeting. A necessity for making a meaningful evaluation of all of these reports is reliable information regarding the acreages of cotton planted in those counties with Bt corn and/or Bt cotton. These data will not be available from the USDA National Agricultural Statistics Service until June 13, 1997. It will take the Agency a few months, from the time the material is submitted, to conduct an evaluation, write the report, and then plan and organize the public meeting.

The Agency believes that, absent a thorough analysis of the situation, suspension of the current Bt-corn and Bt-cotton registrations and delaying the registration of any additional Bt crops would be a precipitous action. EPA will consider the value of such actions only after we have obtained and analyzed pertinent information. Agency scientists communicate frequently with academicians, extension personnel, and commodity groups regarding resistance management practices and experiences in the field. In addition, registrants of Bt crops are required by EPA to report confirmed insect resistance within 30 days and take corrective action after consultation with EPA. Should any of this information demonstrate that a regulatory actions are necessary, EPA will take appropriate actions regarding the registrations of Bt corn and Bt cotton products.

A subpanel of the independent FIFRA SAP met on March 1, 1995 to discuss in part, resistance management of Bt crops. Although that meeting focused primarily on Bt potatoes, the FIFRA SAP subpanel also discussed Bt corn and Bt cotton. The subpanel members recommended that in order to refine existing resistance management plans, large scale use of these plant-pesticides was needed. The Agency agrees with this approach and is allowing such large-scale use, with appropriate safeguards to protect against the development of resistance, while requiring registrants to conduct research necessary to develop acceptable long-term resistance management plans.

Moreover, the Agency has raised, in general, issue of pesticide resistance management to its Pesticide Program Dialog Committee (PPDC). The PPDC supports EPA's continued efforts to protect the use of Bt foliar pesticides and plant-pesticides.

In the interim, EPA has announced a public hearing on March 21, 1997 to solicit comments on resistance management plans for plant pesticides, including the necessity for such plans, criticial elements of resistance management plans and requirements for successful implementation. A follow-up meeting will be held in Texas in late April or May.

C. Response to van Duyn Letter Regarding the Effects of *Bt* Corn Producing Toxin in the Silks and Kernels on the Corn Earworms

On April 22, 1996 Dr. John Van Duyn, North Carolina Cooperative Extension Service, N.C. State University sent a letter to the Agency in regard to restricting the use of *Bt* corn in areas that also grow cotton (or vice-versa). He concludes that sales should not be restricted because there are adequate refugia provided by alternate crops and weeds that would be provide alternate hosts for European corn borer (ECB) and CEW (corn earworm, also known as the bollworm with the scientific name of Helicoverpa zea (Boddie)) other than just corn or cotton.

While EPA agrees that market driven refugia, alternate crops, and weed hosts will provide adequate refugia in the corn belt for the duration of this registration, EPA does not agree that alternate crops and weed hosts provide adequate refugia in cotton growing states. Section IV. E. Resistance Management of this document discusses the risk and benefit considerations made by the Agency which will allow for limited use of this product in cotton growing states. Dr. Van Duyn's letter, along with other public comments and registrant submissions were included in the Agency's registration decision.

IV. SCIENCE ASSESSMENT

The discussion that follows summarizes BPPD's reviews of the data available to the Agency on these plant-pesticide active ingredient. A more detailed discussion of this assessment is provided in the Data Evaluation Records for the studies summarized below.

Dekalb's Corn Line DBT418

Dekalb's corn line DBT418 was transformed with three plasmids which potentially allowed for the incorporation and expression of the *bar*, *pinII* and *cryIA(c)* genes. The southern hybridization analyses of DBT418 indicate the presence of approximately 2 copies of the *cryIA(c)* and *bar* genes and a partial copy of the *pinII* gene. The results of probing the various genomic digests of DBT418 also indicated the single *pinII* gene present was an incomplete copy and one of the *bar* genes was rearranged. Data showed that over 96% of the 190 progeny tested from the generation of elite lines yielded DNA patterns as expected from a stable insertion event for the *cryIA(c)* gene.

A. Human Health

These data and conclusions not only apply to the subject plant-pesticide, but also apply to all plant-pesticides covered by the attached exemptions from the requirement of a tolerance.

1. CryIA(c)

The data submitted regarding potential health effects of CryIA(c) include information on the characterization of the expressed protein in corn, the acute oral toxicity of CryIA(c), and *in*

5822

vitro digestibility studies of the protein. The results of these studies were determined applicable to evaluate human risk and the validity, completeness, and reliability of the available data from the studies were considered. The acute oral toxicity test of bacterially-derived CryIA(c) protein showed no test substance related deaths at a dose of 5000 mg/kg. Although CryIA(c) expression level data was required for an environmental fate and effects assessment, residue chemistry data were not required for a human health effects assessment of the subject plant-pesticides ingredients because of the lack of mammalian toxicity. Both (1) available information concerning the dietary consumption patterns of consumers (and major identifiable subgroups of consumers including infants and children) and (2) safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food additives, are generally recognized as appropriate for the use of animal experimentation data were not evaluated because the lack of mammalian toxicity at high levels of exposure demonstrate the safety of the product at levels above possible maximum exposure levels. This is similar to the Agency position regarding toxicity and the requirement of residue data for the microbial *Bacillus thuringiensis* products from which this plant-pesticides was derived. [See 40 CFR Sec. 158.740(b).] For microbial products, further toxicity testing to verify the observed effects and clarify the source of the effects (Tiers II & III) and residue data are triggered by significant acute effects in studies such as the mouse oral toxicity study.

The acute oral toxicity data submitted support the prediction that the CryIA(c) protein would be non-toxic to humans. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels [Sjoblad, Roy D., *et al.* "Toxicological Considerations for Protein Components of Biological Pesticide Products," Regulatory Toxicology and Pharmacology 15, 3-9 (1992)]. Therefore, since no effects were shown to be caused by the plant-pesticides, even at relatively high dose levels, the CryIA(c) delta-endotoxin protein is not considered toxic.

Adequate information was submitted to show that the CryIA(c) test material derived from microbial cultures were biochemically and, functionally similar to the proteins produced by the plant-pesticides ingredients in corn. Production of microbially produced protein was chosen in order to obtain sufficient material for testing. In addition, the *in vitro* digestibility studies indicate the proteins would be rapidly degraded following ingestion.

The genetic material necessary for the production of the plant-pesticides active and inert ingredients are the nucleic acids (DNA) which comprise (1) genetic material encoding these proteins and (2) their regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the proteins, such as promoters, terminators, and enhancers. DNA is common to all forms of plant and animal life and the Agency knows of no instance where these nucleic acids have been associated with toxic effects related to their consumption as a component of food. These ubiquitous nucleic acids as they appear in the subject active ingredient have been adequately characterized by the applicant. Therefore, no mammalian toxicity is anticipated from dietary exposure to the genetic material necessary for the production of the subject active and inert plant pesticidal ingredients.

Sensitivity of Subgroups

The Agency has considered available information on the variability of the sensitivities of major identifiable subgroups of consumers including infants and children and the physiological differences between infants and children and adults and the physiological differences between infants and children and adults and effects of *in utero* exposure to the plant-pesticides. Since CryIA(c) is a protein, allergenic sensitivities were considered. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases, are glycosylated and present at high concentrations in the food. Data has been submitted which demonstrates that the CryIA(c) delta-endotoxin is rapidly degraded by gastric fluid *in vitro* and is non-glycosylated. Studies submitted to EPA done in laboratory animals have not indicated any potential for allergic reactions to *B. thuringiensis* or its components, including the delta-endotoxin in the crystal protein. Despite decades of widespread use of *Bacillus thuringiensis* as a pesticide (it has been registered since 1961), there have been no confirmed reports of immediate or delayed allergic reactions to the delta-endotoxin itself despite significant oral, dermal and inhalation exposure to the microbial product. Several reports under FIFRA § 6(a)2 have been made for various *Bacillus thuringiensis* products claiming allergic reactions. However, the Agency determined these reactions were not due to *Bacillus thuringiensis* itself or any of the Cry toxins. Thus, the potential for the CryIA(c) protein to be a food allergen is minimal.

Cumulative Effects

The Agency has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Because there is no indication of mammalian toxicity to these plant-pesticides, there are no cumulative effects.

Aggregate Exposures

The Agency has considered available information on the aggregate exposure levels of consumers (and major identifiable subgroups of consumers) to the pesticide chemical residue and to other related substances. These considerations include dietary exposure under the tolerance exemption and all other tolerances or exemptions in effect for the plant-pesticides chemical residue, and exposure from non-occupational sources. Exposure via the skin or inhalation is not likely since the plant-pesticides are contained within plant cells which essentially eliminates these exposure routes or reduces these exposure routes to negligible. Oral exposure, at very low levels, may occur from ingestion of processed corn products and drinking water. However a lack of mammalian toxicity and the digestibility of the plant-pesticides has been demonstrated. The use sites for CryIA(c) delta endotoxin are all agricultural for control of lepidopteran insects. Therefore, exposure via residential or lawn use to infants and children is not expected. Even if negligible exposure should occur, the Agency concludes that such exposure would present no risk due to the lack of toxicity.

7822

FFDCA section 408 provides that EPA shall apply an additional tenfold margin of exposure (safety) for infants and children in the case of threshold effects to account for pre- and post-natal toxicity and the completeness of the database unless EPA determines that a different margin of exposure (safety) will be safe for infants and children. In this instance EPA believes there is reliable data to support the conclusion that the plant-pesticides are not toxic to mammals, including infants and children, and thus there are no threshold effects. As a result, the provision requiring an additional margin of exposure does not apply.

Endocrine Effects

EPA does not have any information regarding endocrine effects for these kinds of pesticides at this time. The Agency is not requiring information on the endocrine effects of these plant-pesticides at this time; and Congress allowed 3 years after August 3, 1996, for the Agency to implement a screening and testing program with respect to endocrine effects.

Conclusion

There is a reasonable certainty that no harm will result from aggregate exposure to the United States population, including infants and children, to the CryIA(c) protein and the genetic material necessary for its production. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. We have arrived at this conclusion because, as discussed above, no toxicity to mammals has been observed for the plant-pesticides. As a result, attached for your signature is an exemption from tolerance requirements pursuant to FFDCA section 408(j)(3) for *Bacillus thuringiensis* CryIA(c) delta-endotoxin and the genetic material necessary for its production in all plants.

Bacillus thuringiensis subspecies *kurstaki* CryIA(c) delta-endotoxin and the genetic material necessary for its production in all plants are exempt from the requirement of a tolerance when used as plant-pesticides in all plant raw agricultural commodities. "Genetic material necessary for its production" means the genetic material which comprise (1) genetic material encoding the CryIA(c) delta-endotoxin and (2) its regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the CryIA(c) delta-endotoxin, such as promoters, terminators, and enhancers.

2. PAT

The data submitted regarding potential health effects of PAT include information on the characterization of the expressed protein in corn, the acute oral toxicity of PAT, and *in vitro* digestibility studies of the protein. The results of these studies were determined applicable to evaluate human risk and the validity, completeness, and reliability of the available data from the studies were considered. The acute oral toxicity test of bacterially-derived PAT protein showed no test substance related deaths at a dose of 2500 mg/kg. Residue chemistry data were not required for a human health effects assessment of the subject plant-pesticide inert ingredients because of the lack of mammalian toxicity. Both (1) available information

8822

concerning the dietary consumption patterns of consumers (and major identifiable subgroups of consumers including infants and children) and (2) safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food additives, are generally recognized as appropriate for the use of animal experimentation data were not evaluated because the lack of mammalian toxicity at high levels of exposure demonstrate the safety of the product at levels above possible maximum exposure levels. This is similar to the Agency position regarding toxicity and the requirement of residue data for the microbial *Bacillus thuringiensis* products from which this plant-pesticides was derived. [See 40 CFR Sec. 158.740(b).] For microbial products, further toxicity testing to verify the observed effects and clarify the source of the effects (Tiers II & III) and residue data are triggered by significant acute effects in studies such as the mouse oral toxicity study.

The acute oral toxicity data submitted support the prediction that the PAT protein would be non-toxic to humans. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels [Sjoblad, Roy D., *et al.* "Toxicological Considerations for Protein Components of Biological Pesticide Products," Regulatory Toxicology and Pharmacology 15, 3-9 (1992)]. Therefore, since no effects were shown to be caused by the plant-pesticides, even at relatively high dose levels, the PAT delta-endotoxin protein is not considered toxic.

Adequate information was submitted to show that the PAT test material derived from microbial cultures were biochemically and, functionally similar to the proteins produced by the plant-pesticides ingredients in corn. Production of microbially produced protein was chosen in order to obtain sufficient material for testing. In addition, the *in vitro* digestibility studies indicate the proteins would be rapidly degraded following ingestion.

The genetic material necessary for the production of the plant-pesticides active and inert ingredients are the nucleic acids (DNA) which comprise (1) genetic material encoding these proteins and (2) their regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the proteins, such as promoters, terminators, and enhancers. DNA is common to all forms of plant and animal life and the Agency knows of no instance where these nucleic acids have been associated with toxic effects related to their consumption as a component of food. These ubiquitous nucleic acids as they appear in the subject active ingredient have been adequately characterized by the applicant. Therefore, no mammalian toxicity is anticipated from dietary exposure to the genetic material necessary for the production of the subject active and inert plant pesticidal ingredients.

Sensitivity of Subgroups

The Agency has considered available information on the variability of the sensitivities of major identifiable subgroups of consumers including infants and children and the physiological differences between infants and children and adults and the physiological differences between infants and children and adults and effects of *in utero* exposure to the plant-pesticides. Since PAT is a protein, allergenic sensitivities were considered. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and

proteases, are glycosylated and present at high concentrations in the food. Data has been submitted which demonstrates that the PAT protein is rapidly degraded by gastric fluid *in vitro* and is non-glycosylated. Thus, the potential for the PAT protein to be a food allergen is minimal.

Cumulative Effects

The Agency has considered available information on the cumulative effects of such residues and other substances that have a common mode of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Because there is no indication of mammalian toxicity to these plant-pesticides , there are no cumulative effects.

Aggregate Exposures

The Agency has considered available information on the aggregate exposure levels of consumers (and major identifiable subgroups of consumers) to the pesticide chemical residue and to other related substances. These considerations include dietary exposure under the tolerance exemption and all other tolerances or exemptions in effect for the plant-pesticides chemical residue, and exposure from non-occupational sources. Exposure via the skin or inhalation is not likely since the plant-pesticides are contained within plant cells which essentially eliminates these exposure routes or reduces these exposure routes to negligible. Oral exposure, at very low levels, may occur from ingestion of processed corn products and drinking water. However a lack of mammalian toxicity and the digestibility of the plant-pesticides has been demonstrated. At present, the use sites for PAT are all agricultural. Therefore, exposure via residential or lawn use to infants and children is not expected. Even if negligible exposure should occur, the Agency concludes that such exposure would present no risk due to the lack of toxicity.

FFDCA section 408 provides that EPA shall apply an additional tenfold margin of exposure (safety) for infants and children in the case of threshold effects to account for pre- and post-natal toxicity and the completeness of the database unless EPA determines that a different margin of exposure (safety) will be safe for infants and children. In this instance EPA believes there is reliable data to support the conclusion that the plant-pesticides are not toxic to mammals, including infants and children, and thus there are no threshold effects. As a result, the provision requiring an additional margin of exposure does not apply.

Endocrine Effects

EPA does not have any information regarding endocrine effects on these kinds of pesticides at this time. The Agency is not requiring information on the endocrine effects of these plant-pesticides at this time; and Congress allowed 3 years after August 3, 1996, for the Agency to implement a screening program with respect to endocrine effects.

Conclusion

There is a reasonable certainty that no harm will result from aggregate exposure to the United States population, including infants and children, to the PAT protein and the genetic material necessary for its production. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. We have arrived at this conclusion because, as discussed above, no toxicity to mammals has been observed for the plant-pesticides. As a result, attached for your signature is an exemption from tolerance requirements pursuant to FFDCA section 408(j)(3) for PAT and the genetic material necessary for its production in all plants.

Phosphinothiricin Acetyltransferase (PAT) and the genetic material necessary for its production in all plants are exempt from the requirement of a tolerance when used as plant-pesticide inert ingredients in all plant raw agricultural commodities. "Genetic material necessary for its production" means the genetic material which comprise (1) genetic material encoding the PAT protein and (2) its regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the PAT protein, such as promoters, terminators, and enhancers.

B. Gene Flow Potential

1. Potential for Outcrossing and Weediness

Although corn is thought to have descended from a wild weedy species, corn today cannot exist in the wild as a weed because the female inflorescence, or the ear, restricts seed dispersal. Corn is an open pollinating (cross-fertilizing) species, probably descended from teosinte, which is more weedy, has more tillers, and does not have ears, as such.

2. Potential for Outcrossing with Wild Corn Species

a. *Teosinte*

Like corn, teosinte also has 10 chromosomes, is wind (open) pollinated, and tends to outcross, but is a highly variable species genetically compatible and interfertile with corn. Corn and compatible species of teosinte freely hybridize when in proximity to each other. In Mexico and Guatemala, teosinte exists as a weed around the margins of corn fields. A frequency of one F1 hybrid (corn x teosinte) for every 500 corn plants, or 2-5% of the teosinte population, has been reported. The F1 hybrid is robust, fertile, and capable of backcrossing to corn. However, except for special plantings, teosinte is not present in the U.S. Its natural distribution is limited to Mexico, and Guatemala.

b. *Tripsacum*

Tripsacum/corn hybrids have not been observed in the field, but have been accomplished in

11822

the laboratory using special techniques under highly controlled conditions. The risk of *Tripsacum*/corn hybrids in the field is considered minimal. Tripsacum/teosinte hybrids have not been able to be produced. Tripsacum species are perennials and seem more closely related to the genus *Manisurus* than either corn or teosinte. *Tripsacum*/corn offspring, when they occur, display various levels of sterility. Of the 16 species of *Tripsacum* described, one is native to the southern tip of Florida, 12 are native to Mexico and Guatemala, and 3 are native to South America.

3. Potential for Outcrossing with Cultivated Zea Varieties

Corn pollen has been shown to travel up to 2 miles under favorable wind conditions. All corns will interpollinate except for certain popcorn varieties. Corn pollen germinates almost immediately after pollination and completes fertilization within 24 hours. Thus corn pollen is highly promiscuous and certification standards for distances between different corn genotypes have been established to maintain desired levels of purity in the production of hybrid corn (a minimum of 660 feet).

4. Weediness of Corn

Transformation causes no change in a corn plant's inability to exist as a weed. Likewise, the ability to outcross with teosinte and tripsacum (under carefully controlled conditions) will not be changed. 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. However, no such case has been known or reported in the U.S.

Gene exchange between cultivated corn and transformed corn would be similar to what naturally occurs at the present time within cultivated corn. 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.

C. Environmental Fate

1. Expression of CryIA(c) Protein in Transformed DBT418 Corn Lines

a. CryIA(c) and PAT protein expression was detected in multiple corn tissues throughout plant development in three different genotypes containing the DBT418 insertion event.

b. The highest levels of CryIA(c) protein were found in leaf tissue, at harvest, ranging from 459.6 to 1198.4 ng CryIA(c)/g dry weight in samples of the three genotypes. CryIA(c) levels did not decline at senescence, reaching a maximum at harvest; however, PAT protein declined during later stages of plant development. This level of expression in senescent corn plants may impact the development of pest resistance.

c. No CryIA(c) or PAT protein was detected in pollen from DBT418 plants. (See study on *Manduca sexta* below.)

d. The tissue distribution and developmental pattern of CryIA(c) protein expression was similar for all three DBT418 genotypes.

e. PAT protein was expressed at significantly higher levels than the CryIA(c) protein in the corresponding tissues in which it was detected. Highest PAT levels were detected in the leaf at pollen shed (501.8-1099.4 ug/g dry weight.)

f. No evidence of PIN II or beta-lactamase protein expression was observed in DBT418 plants.

2. Maximum Expression Levels in Various Corn Tissues

CryIA(c) protein was detected at maximum levels of 1198 ng/g dry weight in the leaf; 124 ng/g in the stalk; 125 ng/g in the root; 43 ng/g in the kernel; 111 ng/g in the silk; and 147 ng/g in the whole plant at pollen shed. (Whole plant values are not available at the harvest stage, which is estimated to be 7-fold greater than that for whole plants at the pollen shed stage.)

PAT protein was detected at maximum levels of 1099 ug/g dry weight in the leaf; 136 ug/g in the stalk; 95 ug/g in the root; 6 ug/g in the kernel; 133 ug/g in the silk; and 120 ug/g in the whole plant at pollen shed. (Since PAT levels decline at harvest, whole plant values at pollen shed are acceptable as maximum values.)

3. Expression of CryIA(c) in Pollen as Determined by THW Bioassay

No toxic effects, mortality, or weight inhibition were observed for the tobacco horn worm (THW) *Manduca sexta*, fed transgenic pollen versus insects fed pollen from untransformed control plants. This would indicate that CryIA(c) is either not present in pollen from DBT418 corn plants, or present below the limits of detection of the bioassay, which is more sensitive than an alternative immunoassay method. (The LC_{50} in THW is 0.036 ug/ml, several hundred-fold less than that for European corn borer, or corn earworm.)

4. Estimated Half-Life and EEC

The maximum amount of CryIA(c) per acre was calculated to be 1.0 g at the Expected Environmental Concentration (EEC) of 1400 ng/kg soil. This, however, is based upon whole plant CryIA(c) values at the pollen shed stage, at which CryIA(c) levels in the leaf are almost 7-fold less than at harvest. Therefore, the EEC calculated in this report is likely to be a significant underestimate. If one applies a 7-fold correction factor, the EEC would be in the order of 9.8 ug/kg soil.

Studies in the literature and on file with the Agency suggest an estimated half-life of CryIA(c)

degradation in soil, as determined by larval bioassay, to be in the range of 9-20 days for purified protein, and 41 days for plant (cotton) tissue. No soil degradation studies were submitted for CryIA(c) in corn tissue. However, data on CryIA(b) in corn suggest a half life of 8 days for purified CryIA(b) in soil, 1.6 days for corn tissue mixed with soil and 26 days for corn tissue without soil.

D. Ecological Effects

1. Impacts on Non-target Organisms

a. Impacts on Birds - Northern Bobwhite Quail MRID# 43999510

The dietary LC50 value for northern bobwhite exposed to lyophilized DBT418 leaf tissue in the diet was determined to be greater than 200,000 ppm (20% w/w), the single concentration tested. No mortalities or signs of toxicity were noted in any of the treatment or control groups tested. There were no apparent differences in the response of the birds to diets containing lyophilized DBT418 leaf tissue when compared to either lyophilized control leaf tissue or basal diet. The CryIA(c) level in the lyophilized DBT418 leaf tissue was 150.5 ng/g dry weight. Insecticidal activity was confirmed by tobacco hornworm bioassay. The level of PAT in lyophilized DBT418 leaf tissue was 209.8 ug/g dry weight. 200,000 ppm lyophilized transgenic leaf tissue is calculated to be approximately equivalent to 4.2 g dry wt. grain/day, or 190.9 g dry wt. grain/kg body weight/day.

b. Impacts on Non-Target Soil Insect - Collembola MRID# 43999512

Chronic (28-day) exposure to 2.0 and 8.0 g/kg lyophilized transgenic (DBT418) leaf tissue and 0.1 mg/kg purified CryIA(c) toxin yielded no treatment-related effects relative to survival and reproduction of *Folsomia candida*. However, overall survival was reduced in all treatment and corresponding control groups relative to untreated controls. Reproduction levels were comparable in all groups, both treated and untreated test and control groups.

The Estimated Environmental Concentration (EEC) was based upon whole plants at the pollen-shed stage; however, data indicate that CryIA(c) levels in the leaf are approximately 7-fold greater at harvest. Applying this correction factor to estimate the total contribution of 1 acre of corn plants at the harvest stage, to the top 15 cm of soil, the EEC would be in the order of 9.8 ug/kg, and the two lyophilized leaf tissue treatment groups in this study would represent 0.03 and 0.13 times the EEC. The 0.1 mg purified toxin treatment group would represent approximately 10 times the EEC.

c. Impacts on Earthworm MRID# 43999513

The LC_{50} was estimated to be greater than 2.0 g/kg DBT418 lyophilized leaf + 0.10 mg/kg CryIA(c) protein, the highest treatment level tested. The NOEC was determined to be 2.0 g/kg DBT418 lyophilized leaf + 0.10 mg/kg CryIA(c) protein.

14822

The (EEC) was based upon whole plants at the pollen-shed stage. However, data indicate that CryIA(c) levels in the leaf are approximately 7-fold greater at harvest. Applying this correction factor to estimate the total contribution of 1 acre of corn plants at the harvest stage, to the top 15 cm of soil, the EEC would be in the order of 9.8 ug/kg, and the two lyophilized leaf tissue treatment groups in this study would represent 0.03 and 0.13 times the EEC. The 0.1 mg purified toxin treatment group would represent approximately 10 times the EEC.

d. Impacts on Fish MRID# 43999514

CryIA(c) activity was shown to be destroyed following the extrusion process utilized in a typical fish food manufacturing process. Thus little or no exposure to cultured fish is expected from commercial preparations of fish food, and further fish studies are waived.

2. Waiver Rationales

Since CryIA(c) is not expressed in the pollen of DBT418 transgenic corn, studies on honeybees and aquatic invertebrates (*Daphnia magna*) are waived.

3. Impacts on Endangered Species

A Biological Opinion was issued on December 18, 1986, concerning the possible effect of foliar spray of *Bacillus thuringiensis* subsp. *kurstaki* (Bt) on threatened and endangered species. Based on the difference in exposure scenarios between foliar Bt spray and Bt delta endotoxin expressed in corn plants, EPA believes that the Biological Opinion is not applicable and that reinitiation of consultation is not required.

The primary route of exposure to foliar Bt sprays is through either direct application to the crop or as a result of drift from spray or aerial applications. In comparison, the primary route of exposure to Bt delta endotoxin in corn is through ingestion of corn tissue. There are no reports of threatened or endangered species feeding on corn plants, thus such species would not be exposed to corn tissue containing the CryIA(c) delta endotoxin. Corn is widely grown, and above ground feeding damage is easily observed on corn plants due to its morphology and cultural practices. Since CryIA(c) protein is not expressed in the pollen of the DBT418 corn line, exposure to soil and aquatic organisms is not expected to occur via this route.

In addition, EPA does not expect that any threatened or endangered species will be affected by outcrossing to wild relatives or by competition with such entities. Hybrid corn cannot exist in the wild, nor are there wild relatives that can interbreed with corn in the United States.

Because EPA expects that threatened or endangered Lepidopteran insects and other species will not be exposed to Bt delta endotoxin, and because the most probable exposure scenario does not appear to affect listed species, EPA believes that this action will have no effect on any threatened or endangered species.

E. Resistance Management

With Dekalb's resistance management plan there are two major resistance concerns for the primary target pest, ECB, and a secondary pest, corn earworm (CEW) [also known as the bollworm, sorghum headworm, tomato fruitworm, and soybean pod borer (*Helicoverpa zea* (Boddie))] . Dekalb's *Bt* corn is similar to NK's and Monsanto's previously registered *Bt* corn product because they all express CryI delta-endotoxins in kernels. CEW prefer to feed upon silks and kernels, although they also feed in the whorl. The CEW can also migrate long distances, can move from corn to cotton and other crops and weed plants, and will move from the South to the North on prevailing winds as the season progresses. Therefore, the Agency believes that there may be similar selection pressure for resistant CEW by Dekalb's *Bt* corn as there is with NK's *and Monsanto's Bt* corn. Additional mitigation measures over that required for Ciba and Mycogen *Bt* corn products are recommended to limit the selection pressure for CEW resistance especially in the southern U.S.

F. Public Interest Finding

DeKalb submitted information pursuant to a finding of public interest in documentation submitted to the Agency on February 24, 1997. The criteria for Agency evaluation of public interest findings is outlined in 51CFR No. 43, Wednesday March 5, 1986. The proposed product clearly does not qualify for the automatic presumptive finding under part IV.A of that document. DEKALB Bt corn is not for minor use, is not a unique replacement for pesticides of concern, and is not for use against a public health pest. Therefore the product must qualify under the criteria outlined in IV B.

Like other Bt corn plant-pesticides, DEKALB Bt corn provides the benefit of reduced chemical pesticide use and the associated human health and environmental risks that go along with using more toxic chemical pesticides registered to control ECB including: carbaryl, chlorpyrifos, carbofuran, fonofos, permethrin, esfenvalerate, methyl parathion, terbufos, and phorate. In addition to the benefits of other Bt corn plant-pesticides, DEKLAB Bt corn shows higher comparative yields to certain other Bt corn varities and demonstrated economic benefits compared to chemical sprays in the data submited. Furthermore, the DEKALB Bt corn meets the criteria in IV B. in that the unique corn varieties offered by this registration have specific advantages related to the need factors described in the FRN. These varieties offer special characteristics not available to growers with respect to adaptation to soil type, maturities, climate adaptation and geographic suitability (see usage factors also). Performance factors have been adequately addressed by the submitted data, and these varieties have been shown to be efficacious in field use.

For the above reasons, the submitted information satisfy the requirements outlined in the Agency policy on public interestest finding and the conditional registration of DEKALB Bt corn has been determined to be in the public interest.

V. TERMS AND CONDITIONS OF THE REGISTRATION

The following listing gives the terms and conditions of the amendment agreed to by Dekalb.

1. This registration will automatically expire on midnight April 1, 2001. EPA will reevaluate the effectiveness of Dekalb's resistance management plan before April 1, 2001 on whether to convert the registration to a non-expiring registration.

2. This registration is for field corn only.

3. Dekalb will:

   a. Unless demonstrated to EPA's satisfaction that alternative resistance management practices are equally or more effective than a structured refugia, develop and submit to EPA a draft plan for "structured" refugia by 8/9/98 and a final plan by 1/31/99;

   b. Discuss the development and implementation of the plan and alternative resistance management practices with EPA throughout development and implementation; and

   c. Implement the "structured" refugia plan or an EPA approved alternative resistance management plan no later than April 1, 2001.

4. Dekalb will monitor for the development of resistance using baseline susceptibility data and/or a discriminating concentration assay when such an assay is available. Dekalb will proceed with efforts to develop a discriminating concentration assay. Dekalb will ensure that monitoring studies are conducted annually to determine the susceptibility of ECB and corn earworm (CEW) populations to the CryIA(c) protein. This resistance monitoring program will be developed to measure increased tolerance to *Bt* corn above the various regional baseline ranges. Annual reports of resistance monitoring will be submitted to the Agency by January 31 each year and include units sold per state of the Dekalb European corn borer protected corn. Lepidoptera pest resistance, for CEW, will be monitored in areas where ECB resistance is being investigated.

Populations of ECB and CEW will be collected from representative distribution areas of the registrant's Bt corn hybrids and monitored/screened for resistance, with particular focus on those areas of highest distribution in each State. The variability of ECB populations and their sensitivity to CryIA(c) was not demonstrated in Dekalb's submission although it is referred to in passing. In the first annual report, Dekalb should indicate where this variability is found and if it has changed with deployment of DBT418. The results of monitoring studies will be communicated to the Agency on an annual basis, by January 31 of the year following the population collections for a given growing season.

In addition, Dekalb will instruct its customers (growers and seed distributors) to contact Dekalb (e.g., via a toll-free customer service number) if incidents of unexpected levels of ECB & CEW damage occur. Dekalb will investigate and identify the cause for this damage by local field sampling of plant tissue from its hybrids and sampling of ECB & CEW populations, followed by appropriate *in vitro* and *in planta* assays. Upon Dekalb's confirmation by immunoassay that the plants contain CryIA(b) protein, bioassays will be conducted to determine whether the collected ECB population exhibits a resistant phenotype.

Until such time that a discriminating concentration assay is established and validated by Dekalb, Dekalb will utilize the following to define a confirmed instance of ECB & CEW resistance:

Progeny from the sampled ECB or CEW population will exhibit both of the following characteristics in bioassays initiated with neonates:

a. An LC50 in a standard CryIA(c) diet bioassay that exceeds the upper limit of the 95% confidence interval of the mean historical LC50 for susceptible ECB or CEW populations, as established by the ongoing baseline monitoring program.

b. Upon validation of the assay, a specified % survival and %leaf area damaged in a 5-day bioassay using CryIA(c)-positive leaf tissue under controlled laboratory conditions.

Based upon continued experience and research, this working definition of confirmed resistance may warrant further refinement. In the event that Dekalb finds it appropriate to alter the criteria specified in the working definition, Dekalb must obtain Agency approval in establishing a more suitable definition.

5. Dekalb will report all instances of confirmed ECB & CEW resistance, as defined above, to the Agency within 30 days. Upon identification of a confirmed instance of ECB resistance Dekalb will take the following immediate mitigation measures:

a. Notify customers and extension agents in the affected area,

b. Recommend to customers and extension agents in the affected area the use of alternative control measures to reduce or control the local ECB population, and

c. Recommend to customers and extension agents in the affected area that crop residues be incorporated into the soil following harvest, to minimize the possibility of overwintering of ECB.

Within 90 days of a confirmed instance of ECB and/or CEW resistance, as defined above, Dekalb will: (1) notify the Agency of the immediate mitigation measures that were implemented, and (2) submit to the Agency a proposed long-term resistance management

action plan for the affected area, (3) work closely with the Agency in assuring that an appropriate long-term resistance management action plan for the affected area is implemented, and (4) implement an action plan that is approved by EPA and that consists of some or all the following elements, as warranted:

a. Informing customers and extension agents in the affected area of ECB and/or CEW resistance,

b. Increasing monitoring in the affected area, and ensuring that local ECB populations are sampled on an annual basis,

c. Recommending alternative measures to reduce or control ECB or CEW populations in the affected area,

d. Implementing a structured refuge strategy in the affected area based on the latest research results. The implementation of such a strategy will be coordinated by the Agency with other registrants.

e. If the above elements are not effective in mitigating resistance, Dekalb will voluntarily cease sale of all of Dekalb's CryIA(c) corn in the county experiencing loss of product efficacy and the bordering counties until an effective local management plan approved by EPA has been implemented. During the voluntary suspension period, Dekalb may sell and distribute in these counties only by obtaining EPA approval to study resistance management in those counties. The implementation of such a strategy will be coordinated by the Agency with other registrants.

If EPA agrees that an effective resistance management plan has been implemented which mitigates resistance, Dekalb can resume sales in the affected county(ies).

6. Dekalb will maintain a (confidential) database to track its sales (units and location) of its Bt corn on a county-by-county basis, to the extent that such data are available. Dekalb will provide annually, on a CBI basis, sales data for each state indicating the number of units of it Bt corn hybrids that it sells. This information will be provided by January 31 of the year following each growing season.

7. Dekalb will provide grower education. Dekalb will agree to include an active partnership with such parties as: university extension entomologists and agronomists, consultants, and corn grower groups. Dekalb will implement a grower education program directed at increasing grower awareness of resistance management, in order to promote responsible product use. As specific resistance management recommendations are developed (e.g., as a result of ongoing research or experience) these will be incorporated, as appropriate, into the various grower communication and educational media. Dekalb will inform the Agency as it develops, implements, and refines its communication strategies. In addition to grower

communication vehicles, Dekalb will also develop a Grower Guide, to be distributed to all customers, that will include current information regarding resistance management and integrated pest management.

8. Dekalb will confer with the EPA as Dekalb develops various aspects of its resistance management research program. Dekalb agrees, as a condition of this registration, to submit annually progress reports including results and conclusions from research (including literature) as they become available in the following areas as a basis for developing a long-term resistance management strategy which include:

a. ECB pest biology and behavior including adult movement and mating patterns, larval movement, survival on silks, kernels, and stalks, and overwintering survival and fecundity on non-corn hosts.

b. The feasibility of "structured" refuge options for ECB including both "block" refugia, "50-50 early/late season patchwork"; research needs to be done in both northern and southern areas on ECB.

c. Development of a discriminating concentration (diagnostic concentration) assay for field resistance (field screening) for ECB and CEW. Specific sampling locations will be established in each state to determine if increases in *Bt* toxin tolerance may be detected before crop failures develop. Increased tolerance levels need to be identified before field failure occurs. In monitoring for tunneling damage, the number of trivial tunnels may be less indicative of resistance development than the total extent of tunneling damage (e.g. length of tunnels). The extent of tunneling damage should be monitored as well as the number of tunnels.

d. Effects of corn producing the CryIA(c) delta endotoxin on pests other than ECB, including but not limited to the corn earworm, the stalk borer complex, and the fall armyworm. Dekalb should provide information to support their claim of CEW control from foliar feeding.

e. The biology of ECB resistance including receptor-mediated resistance and its potential effect on population fitness, as well as the effects on insect susceptibility to other Cry proteins. Possible high dose control exists for the first generation ECB on whorl stage, but not for later generation(s) on more mature corn plants. More data are needed on toxin expression in various parts of the plant at different stages of the plant in regard to ECB and CEW. As part of this data, Dekalb must provide information in silk expression levels and on any reduced CEW reproduction offered by DBT418 from field data. Data will also be required of other secondary pests of corn (i.e. stalk borer complex, fall armyworm, and S.W. corn borer) if EPA determines that research in Section 8.d. above so warrants.

9. Dekalb may not sell or distribute any corn that contains the plant-pesticide product covered by this document in counties of the following states as listed:

Alabama	all
Arkansas	all
Florida	all
Georgia	all
Louisiana	all
Mississippi	all
Missouri:	Butler, Dunkin, Mississippi, New Madrid, Pemiscot, Scott, Stoddard Counties.
Oklahoma:	Bryan, Caddo, Canadian, Garvin, Grady Counties.
North Carolina	all
South Carolina	all
Tennessee:	Carroll, Chester, Crockett, Fayette, Franklin, Gibson, Hardeman, Hardin, Haywood, Henderson, Lake, Lauderdale, Lawrence, Lincoln, McNairy, Madison, Obion, Rutherford, Shelby, Tipton Counties.
Texas	all
Virginia:	Greensville, Isle of Wright, Northampton, Southampton, Sussex, Suffolk Counties.

Dekalb will develop a resistance management program that is acceptable to EPA that includes the research specified in Section 8.a. through e. above.

VI. RATIONALE FOR RECOMMENDATION

Pursuant to FIFRA section 3(c)(7)(C), EPA may conditionally register a new pesticide active ingredient if: 1) insufficient time has elapsed since the imposition of the data requirement for those data to be developed and all other required data have been submitted, 2) the use of the pesticide product during the period of the conditional registration will not cause any unreasonable adverse effect on the environment, and 3) the registration and use of the pesticide during the conditional registration is in the public interest. BPPD believes that all these criteria have been fulfilled.

The first criteria under FIFRA section 3(c)(7)(C) mentioned above has been met since insufficient time has elapsed since the imposition of the data requirements for insect resistance management.

The applicant has submitted or cited data to satisfy the second criterion for conditional registration under FIFRA 3(c)(7)(C) as mentioned above. Dekalb has submitted and/or cited satisfactory data pertaining to the proposed use of the product corn. The human health effects

data and nontarget organism effects data are considered sufficient for the period of the conditional registration. These data demonstrate that no forseeable human health hazards or ecological effects are likely to arise from the use of the product and that the risk of resistance developing to *Bacillus thuringiensis* during the conditional registration is not expected to be significant. The data also demonstrates that there is virtually no possibility of any risk associated with weediness or outcrossing to wild relatives. In view of these minimal risks and the benefits (1. reduced worker exposure to alternative chemical pesticides including: carbaryl, chlorpyrifos, carbofuran, fonofos, permethrin, esfenvalerate, methyl parathion, terbufos, and phorate; and 2. increased yields compared to non-Bt corn and other Bt corn), BPPD believes that the use of the product during the limited period of the conditional registration will not cause any unreasonable adverse effects.

Although the data with respect to this particular new active ingredient is satisfactory, it is not sufficient to support an unconditional registration under FIFRA 3(c)(5). Additional data is necessary to evaluate the risk posed by the continued use of this product. Consequently, BPPD recommends imposing the data requirements specified earlier in this Decision Document in Section V.

BPPD also believes that the third criterion for a FIFRA 3(c)(7)(C) conditional registration has been fulfilled because the use of Dekalb Bt corn under this registration would be in the public interest.

VII. RECOMMENDATION

The submitted data in support of this registration under section 3(c)(7)(C) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) have been reviewed and determined to be adequate. Studies regarding insect resistance management are included in the terms, conditions, and limitations of this registration. This registration will not cause unreasonable adverse effects to man or the environment and is in the public interest.

Based on the data submitted and cited by the applicant and reviewed by OPP staff, Biopesticides and Pollution Prevention Division recommends that Dekalb's plant-pesticide product containing the active ingredient *Bacillus thuringiensis* CryIA(c) protein and the genetic material necessary for its production in field corn be CONDITIONALLY REGISTERED for use under 3(c)(7)(C) of FIFRA.

CONCUR: _____

NONCONCUR: _____

DATE: 3/25/97

22822