Chemical Code: **006430** Date Out: 5 APR 1993

ENVIRONMENTAL FATE AND GROUND WATER BRANCH

Review Action

To: Phillip Hutton, PM 18
Registration Division (H7505C)

From: Robert W. Pilsucki, Ph.D., Microbiologist
Environmental Chemistry Review Section #1
Environmental Fate & Ground Water Branch/EFED (H7507C)

Thru: Paul J. Mastradone, Ph.D., Chief
Environmental Chemistry Review Section #1
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Henry Jacoby, Chief
Environmental Fate & Ground Water Branch/EFED (H7507C)

Attached, please find the EFGWB review of...

DP Barcode: 187336			
Common Name:	*Bacillus thuringiensis* delta endotoxin expressed in corn (*Zea mays* L.)	**Trade name:**	None
Company Name:	Seed Division of Ciba Geigy Corporation		
ID #:	066736-EUP-R		
Purpose:	Experimental Use Permit		

Type Product:	Action Code:	EFGWB #(s):	Review Time:
Transgenic Plant Pesticide	720	93-0364	8.0 days

STATUS OF STUDIES IN THIS PACKAGE:

Guideline #	MRID	Status[1]

STATUS OF DATA REQUIREMENTS:

	Status[2]

DATA EVALUATION RECORD

Biological Fate ("Pollen Dispersal Studies): Transgenic corn plants producing a delta endotoxin insect control protein from *Bacillus thuringiensis* subsp. *kurstaki;* a phosphinothricin acetyltransferase (PAT) enzyme and/or a beta-glucuronidase (GUS) enzyme marker from *Escherichia coli*. DP Barcode D187336; EFGWB #93-0364.

REVIEWED BY:

Leo R. LaSota, Ph.D
Biologist
EFGWB/EFED

Signature: Leo R. LaSota
Date: 5 APR 1993

APPROVED BY:

Robert W. Pilsucki, Ph.D.
Microbiologist
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Paul Mastradone, Ph.D.
Chief, Section 1
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Signature: Paul J. Mastradone
Date: 5 APR 1993

CONCLUSIONS:

Based on the data submitted, EFGWB believes that the "Pollen Dispersal Studies" are really outcrossing studies and that the applicant's conclusion that "Most of the purple marker pollen did not travel beyond 15 feet (six rows) of its source" is not supported by the data.

Factors affecting the rates of reported "pollen dispersal" include:

1. Number of border rows;
2. Distances from pollen sources to recipient pistils;
3. Genotypes of the pollen and pistil parents;
4. Onset and duration of pollen shed for both parents;
5. Onset and duration of pistil receptivity for target plants; and
6. Climatic conditions, particularly wind speed and temperature, during the period of pollen shed.

Problems with the experimental design make it impossible to determine to what extent each of these factors contributed to the outcrossing. For example:

1. There were six varieties of purple aleurone marker corn and either six (Illinois site) or four (Hawaii site) varieties of "trap" corn used in these experiments. The perfect "nick" was sacrificed in an attempt to provide the longest "nick."

2. The maximum outcrossing rate adjacent to the marker corn at the Illinois site was only 4.91% (North); at the Hawaii site it was only 28.6%. To what extent did the multiple genotypes/phenotypes reduce the possibility for successful outcrossing?

3. Target plants were not detasseled and thus served as pollen sources for self- and outcrossing within the trap rows.

4. Target plant varieties were not randomized within rows, but merely repeated as single varieties in identical row order from the purple aleurone plants.

5. Distance could not be separated from number of trap rows as a variable affecting outcrossing.

RECOMMENDATIONS:

EFGWB has no further recommendations at this time.

MATERIALS AND METHODS:

POLLEN DISPERSAL STUDIES

Background:

"In 1991 Ciba Seeds conducted pollen dispersal studies as part of transgenic corn field experiments at Bloomington, Illinois and Molokai, Hawaii (conducted under USDA/APHIS permits 91-025-01 and 91-100-01, respectively). Part of each plot contained corn with dominant marker genes causing purple kernels. The spread of pollen from these genotypes was measured by sampling grain from border rows and counting purple kernels."

Study Design:

"At the Illinois plot, the transgenic corn and the inbreds for the hybrid crosses constituted the center of the plot. This area was surrounded by six varieties of purple aleurone marker corn, followed by 36 rows of hybrid corn which was used to "trap" pollen from the purple marker plants. The Ciba Seeds hybrids (4393, 4425, 4447, 4626, 4513, 4543) represented four different maturities and provided a 6.5 day range in mid-silk dates. This allowed a broad time range during which silks were receptive. One row of each hybrid was planted per group of six rows, and each group was repeated six time to form the 36 border rows. Rows were planted 30 inches apart; therefore, the last border row was at least 90 feet from the source of purple marker pollen. At harvest, up to fifteen ears were sampled from each row at each of eight compass directions radiating from the center of the plot. Purple kernels were counted and the total number of kernels were estimated for each sample.

The design of the Hawaii study was similar to that of the Illinois study. However, the border rows were arranged differently. On the northeast and southwest sides of the plot, nine groups of four hybrids were planted instead of the six groups of six which were used in Illinois. On the southeast and northwest sides, only one hybrid was planted in a fifteen-foot block, or range, followed by similar blocks of five other hybrids. Also, during the harvest of the Hawaii plot, plants were sampled in only four directions radiating from the plot's center."

REPORTED RESULTS:

Results are summarized by hybrid group in Figures 1 (Illinois) and 2 (Hawaii). For Illinois, the raw data for each row are presented in the Appendix, Tables 1A-1H. Raw data for Hawaii are presented in Appendix Tables 2A-2D.

"Most of the purple marker pollen did not travel beyond 15 feet (six rows) of its source. However, in some cases, there were small numbers of grains which did spread to the edges of the plot. Most dissemination was in the direction of prevailing winds.

In Illinois, percent outcrossing 15 feet from the marker pollen source was 0.15%. At 90 feet from the source, outcrossing decreased to 0.00-0.03%. At the Hawaii location, which typically has very strong winds from the northeast, samples taken from the southwest side of the plot showed 3.1% outcrossing at 20 feet, and 0.47% outcrossing at 90 feet. In the other directions at the Hawaii site, outcrossing did not exceed 0.23% beyond 10-15 feet, or 0.02% at 90 feet from the pollen source."

APPENDICES:

References

Vlachos, D.A. 1993. Experimental use permit request for field testing of *Bacillus thuringiensis* subsp. *kurstaki* CryIA(b) insect control protein as expressed in corn plants. EPA DP Barcode D187336.

Figures and Tables (See Attached)

DATA EVALUATION RECORD

Chemical Exposure: Transgenic corn plants producing a delta endotoxin insect control protein from *Bacillus thuringiensis* subsp. *kurstaki*; a phosphinothricin acetyltransferase (PAT) enzyme and/or a beta-glucuronidase (GUS) enzyme marker from *Escherichia coli*. DP Barcode D187336; EFGWB #93-0364.

REVIEWED BY:

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Date: 5 APR 1993

APPROVED BY:

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Paul Mastradone, Ph.D.
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Signature: Paul J. Mastradone
Date: 5 APR 1993

CONCLUSIONS:

1. Based on the data submitted and a review of the scientific literature, it appears that there is no unreasonable risk of offsite exposure to the CryIA(b) protein for the proposed field tests if pollen is prevented from maturing or is enclosed in protective coverings except during controlled pollen transfer to silks.

2. Based on the data submitted and a review of the scientific literature, it appears that the containment measures proposed and recommended for open-pollinated field tests of transgenic corn(See Biological Fate), can reduce, but not eliminate, offsite exposure to the CryIA(b) gene. With these measures, it is expected offsite expression of the cryIA(b) by pollen transport and gene capture through outcrossing can be reduced to less than one per cent. This assessment applies **only** for field tests to be completed on before March 31, 1994. Insufficient information is available to make a chemical exposure assessment for the field tests proposed to begin on or after April 1, 1994.

RECOMMENDATIONS:

EFGWB recommends that the applicant provide the following additional information concerning the expression levels for the CryIA(b) and marker gene proteins in the transgenic corn:

1. Specific details of method validation pertaining to tissue preparation to determine whether or not the integrity of assayed proteins was maintained during processing;
2. Recovery efficiencies for target proteins and spikes, if any, assayed;
3. Expression levels of the transgenic proteins expressed on a per acre basis (grams/acre) in mature corn plants (maximum biomass) using the highest levels reported for each vector examined at the plant density (i.e. 25,000 plants/acre) indicated for these tests;
4. The percentage of the proposed field tests devoted to corn transformed with tissue specific (pollen and PEPC) or constitutive (CaMV) promoters.

EFGWB also recommends that, during the conduct of the 1993-94 field tests, expression levels of the transgenic proteins be determined for selected lines of field grown plants at points throughout the growing season to monitor changes in transgenic plant pesticide expression as a function of plant maturity.

EFGWB has insufficient information from the applicant to make recommendations concerning chemical exposure for field tests proposed to commence on or after April 1, 1994.

MATERIALS AND METHODS:

The following information is excerpted from Koziel *et al.* (1993):

CryIA(b) protein quantification. Detection and quantitative determination of the amount of CryAI(b) protein expressed in transgenic plants was achieved using enzyme-linked immunosorbant assays (ELISA) as described by Clark *et al* (1986). Immunoaffinity purified polyclonal rabbit and goat antibodies specific for the insecticidal crystal proteins from *Bacillus thuringiensis* subsp. *kurstaki* HD-1 were used to determine ng CryIA(b) per mg soluble protein from crude extracts of leaf samples. The sensitivity of the double sandwich ELISA is 1-5 ng CryIA(b) per mg soluble protein using 50 g of total protein per ELISA microtiter dish well. Corn tissue extracts were prepared by grinding leaf tissue in gauze lined plastic bags using a hand held ball-bearing homogenizer (AGDIA, Elkart, IN.) in the presence of extraction buffer (50 mM Na_2CO_3 pH 9.5, 100mM NaCl, 0.05% Triton, 0.05% Tween, 1 mM phenylmethylsulfonyl fluoride and 1 M leupeptin). Protein

determination was performed using the Bio-Rad (Richmond, CA) protein assay.

Southern blot analysis. Genomic DNA was isolated from maize plants and processed for Southern blot analysis using standard procedures (Sambrook *et al*). A gel purified fragment containing only the synthetic cryIA(b) gene was used to generate a random-primed ^{32}P probe. Southern blots were prepared and hybridized using standard procedures and washed at 65°C in 0.3 X SCC.

REPORTED RESULTS:

Based on the data submitted by the applicant, it appears that:

1. Transgenic plants of corn (*Zea mays* L.) expressing a CryIA(b) insect control protein derived from *Bacillus thuringiensis* subsp. *kurstaki (B.t.k.)* are proposed for field testing on 32.5 acres (104.7 acres including nontransgenic controls, border rows and alleyways) in nine locations in six states for the period ending on or before March 31, 1994.

2. Proposed field tests of corn containing the *B.t.k.* gene for the period April 1994 through March 1995 include 104.1 acres of transgenic plants at 24 sites in 17 states. Details concerning experimental protocols were not submitted for these tests. Consequently, total acreage (including nontransgenic controls, border rows and alleyways) could not be determined for tests proposed to commence on or after April 1, 1994.

3. The maximum CryIA(b) protein in seed planted through March 1994 is expected to be 35.7 grams.

4. The maximum CryIA(b) protein in seed planted from April 1994 through March 1995 is expected to be 112.0 grams. Details concerning experimental protocols were not submitted for these tests. Consequently, the possible increase in total CryIA(b) protein in seed planted from April 1994 through March 1995 from all anticipated sequential plantings could not be calculated.

5. The highest reported level of the CryIA(b) protein in seeds tested was 47 ng/g fresh weight (18 ng/mg soluble protein) for plants transformed with the PEPC/pollen promoter and 712 ng/g fresh weight (274 ng/mg soluble protein) for plants transformed with the CaMV constitutive promoter.

6. On-site exposure to the CryIA(b) protein may occur in roots, pith, leaves, pollen and seeds.

Expression levels for the *B.t.k.* protein will vary according to the cassette inserted, plant transformed, tissue, stage of development and growing conditions at each test site. Levels reported by the applicant are listed in Table 1 of Reported Results. Expression levels for the marker genes were not provided by the applicant for any plant tissue.

Table 1*

Quantification of CrylA(b) protein levels in various tissues of maize. Values are ng CrylA(b)/mg soluble protein ± standard deviation.

	LEAF		ROOT	PITH	POLLEN/ ANTHER	KERNEL
35S LINES						
CG00554 x 171-18	513±244	(n=4)	596	288	NT	53
CG00554 x 171-4A	1732±39	(n=2)	NT	NT	NT	NT
CG00554 x 171-13	767±842	(n=8)	1209	4381	0	274
CG00554 x 171-14A	655±554	(n=7)	3348	2440	0	49
CG00615 x 171-16BB	283±227	(n=4)	74	71	0	NT
PEPC\POLLEN LINES						
CG00661 x 176-10	1703±378	(n=9)	52	60	260	15
CG00554 x 176-11	1288±583	(n=12)	54	113	418	16
CG00642 x 176-10	1138±188	(n=4)	NT	NT	NT	NT
CG00689 x 176-11	1077±108	(n=3)	NT	NT	340	NT
176-11 x CG00526	1842±345	(n=2)	47	53	NT	18

Values for control plants analyzed by ELISA are 0 ng.
Plants from event 171 contain the chimeric CaMV 35S/CrylA(b) gene while plants from event 176 contain the chimeric PEPC/CrylA(b) and pollen-specific/CrylA(b) genes.
NT=not tested.
*Vlachos (1993).

The following information is excerpted from Koziel *et al.* (1993):

"Transgenic plants with the best ECB damage ratings were analyzed for the CryIA(b) protein levels using ELISA. Leaves from the field plants were sampled seven weeks post-transplant. Transgenic plants containing two synthetic cryIA(b) genes driven by the PEPC and pollen specific promoters produced over 1,000 ng CryIA(b)/mg soluble protein at week seven and were shown later in the season to exceed 4,000 ng CryIA(b)/mg soluble protein. CryIA(b) protein levels in certain plants with the CaMV 35S promoter were as

high at week seven as the levels in the PEPC and pollen promoter plants, but overall, these plants typically showed a much greater variation in CryIA(b) levels both within a particular cross with a given genotype and among the genotypes."

References

Clark, M.F., R.M. Lister and M. Bar-Joseph. 1986. ELISA techniques. Methods in Enzymology 118: 742-766.

Koziel, M.G., G.L. Beland, C. Bowman, N.B. Carozzi, R. Crenshaw, L. Crossland, J. Dawson, N. Desai, M. Hill, S. Kadwell, K. Launis, K. Lewis, D. Maddox, K. McPherson, M.R. Meghji, E. Merlin, R. Rhodes, G.W. Warren, M. Wright and S.V. Evola. 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. Bio/Technology 11: 194-200.

Sambrook, J., E.F. Frisch and T. Maniatis. 1989. Molecular cloning: a laboratory manual. Second Edition. Cold Spring Harbor Laboratory Press.

Vlachos, D.A. 1993. Experimental use permit request for field testing of *Bacillus thuringiensis* subsp. *kurstaki* CryIA(b) insect control protein as expressed in corn plants. EPA DP Barcode D187336.

1. **SOURCES OF TRANSGENES:**

A delta endotoxin insect control protein from *Bacillus thuringiensis* subsp. *kurstaki*; a phosphinothricin acetyltransferase (PAT) enzyme and/or a beta-glucuronidase (GUS) enzyme marker from *Escherichia coli*.

2. **TEST MATERIAL:**

Transgenic and nontransgenic plants of cultivated corn, *Zea mays* L.

3. **STUDY/ACTION TYPE:**

Experimental Use Permit.

4. **STUDY IDENTIFICATION:**

Vlachos, D.A. 1993. Experimental use permit request [January 15, 1993] for field testing of *Bacillus thuringiensis* subsp. *kurstaki* CryIA(b) insect control protein as expressed in corn plants. Ceiby-Geigy Corporation, Seed Division ("Ciba Seeds"), 3054 Cornwallis Drive, P.O. Box 12257, Research Triangle Park, North Carolina. EPA DP Barcode D187336. **EFGWB #93-0364.**

5. **REVIEWED BY:**

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7. **CONCLUSIONS:**

Biological Fate

Based on the data submitted and a review of the scientific literature, it appears that the following conclusions can be drawn for this EUP submission:

1. The proposed field tests at the designated sites would pose no unreasonable risk of gene capture and expression of the transgenic plant pesticide by wild or weedy relatives of corn (*Zea mays* L.). See Biological Fate Data Evaluation Record.

There are no sexually compatible wild or weedy relatives of *Zea mays* near any of the sites proposed for these field tests (Gould and Shaw 1983).

2. Containment measures proposed by the applicant are designed to prevent or limit gene capture and expression of the transgenic plant pesticide by cultivated corn growing close to the proposed field tests.

Corn is a wind-pollinated, monoecious species in the grass (Gramineae) family. Separate staminate (tassel) and pistillate (silk) flowers encourage natural outcrossing within and between fields of corn.

An enormous amount of redundant corn pollen is produced for each successful fertilization of an ovule on the ear. Numerous studies have attempted to quantify this production. For example, pollen grains per anther have been reported to range from 2000 (Sturtevant 1881) to 7500 (Goss 1968). Thus, with an average of 7000 anthers per tassel, and 2000 grains per anther, each tassel could produce 14 X 10^6 pollen grains. Kiesselbach (1949) estimated that 42,500 pollen grains are produced per square inch of cornfield.

The ratio of polln grains produced to ovules fertilized has also been estimated. Goss (1968) reported:

> "Since each ear requires about 1000 pollen grains for fertilization, it appears that there are about 20,000 pollen grains per kernel in excess of what is actually needed if pollination were 100 per cent efficient. This large excess of pollen grains is typical of anemophilous plants--those using wind as an agent for pollination--of which corn is an example."

At 500 kernels per ear, and a pollen to kernel ratio from 9000 (Bonnett 1947) to 50,000 (Weatherwax 1955) to 1, an average ear would require the production of from 4.5 to 25 million grains. Thus, even under the most conservative male fecundity estimates, field tests of corn conducted without either emasculating or shielding tassels will introduce a high inoculum of pollen grains to and around the test sites.

3. Depending on the experiment being conducted, the applicant has proposed various strategies to limit offsite exposure of corn to transgenic plant pollen. These measures include: detasseling of transgenic plants before pollen is shed; covering of tassels of transgenic plants prior to pollen shed with waterproof bags; spatially isolating test plots by planting at least 660 feet from other corn; including nontransgenic border rows; temporally isolating test plots by planting at

least two weeks before or after any nearby production fields.

The first two measures, detasseling or covering of tassels, can theoretically prevent the movement of any transgenic pollen beyond the test site. Field monitoring at daily intervals near, but **before**, pollen shedding begins will be necessary to make either strategy of pollen control effective (Wych 1988). Restricting exposure of the covered pollen by conducting controlled crosses during periods of low wind velocity will help assure the effectiveness of bagging.

Isolation and border rows are designed to reduce, but not entirely eliminate, the transgenic pollen transported beyond the test site. The effectiveness of both measures is attributable, in part, to the relatively large size of corn pollen.

Measuring about 0.1 mm (90 to 100) in diameter, corn pollen is considered large compared to the average pollen size for most plant species and is the largest pollen among members of the grass family (Wodehouse 1935). It has also been reported that corn pollen is the largest of *any* pollen normally disseminated by wind from a comparably low level of elevation by Raynor, Ogden and Hayes (1972). These same authors used wind impaction samplers at four heights (0.5 to 4.6 m) to collect corn pollen at various distances from a sweet corn planting and concluded:

> "The dispersion of corn pollen is influenced by its large size and rapid settling rate. At 60 m from the source in the downwind direction, concentrations average about 1% of those at 1 meter. Width of the pollen plume at 60 m is less than that of smaller pollens under similar conditions. The rate of settling opposes the rate of upward dispersion so that the height does increase continuously with downwind distance. At 60 m from the source concentrations integrated in the crosswind direction average from 3 to 6% of those at 1 m. The total amount of pollen remaining airborne at 60 m is 5% of that at 1m. The velocity of deposition at 32 m from the source averages 33 cm/sec (Raynor *et al* 1972)."

These authors monitored both wind speed and pollen shedding during the course of data colection.

In a similar study, Jones and Newell (1946) collected pollen grains on microscope slides at various elevations and distances from a corn plot.

> "Data based on the average of all elevations for the two-year period show 203 pollen grains were caught daily in the center of the field, while to the north 35 were caught at 5 rods, nine at 15 rods, six at 25 rods, two at 40 rods and one at 60 rods. The average of one pollen grain caught [per 16 square mm area of microscope slide] at 60 rods is the equivalent of 40 per square inch or 5,760 per square foot.

Considering this in another light, there are approximately three square inches of silk mass exposed per shoot. On the basis of data obtained from exposed slides, about 120 grains would have fallen on the silk area daily in fields at a distance of 60 rods. Over a 10- to 12-day period of pollination, 1,200 to 1,400 pollen grains would have fallen on the area occupied by the silks of one ear shoot, or per 800 to 1,000 ovules. With no competition this amount would be sufficient to cause fair seed set."

Metereological conditions during these experiments were recorded in the field with an anemometer and hygrothermograph.

Settling out and dispersion will both act to reduce the corn pollen load as distance from the pollen source is increased. The minimum isolation distance proposed for these field tests is 660 feet, the same as that required for the production of foundation corn seed (Anonymous 1971). This certification distance is designed to achieve a high level of purity for the seed produced within the field, not to prevent outcrossing to adjacent fields (Wrubel *et al* 1992).

Haskell and Dow (1951) examined seed set in hybrid corn as affected by the direction and distance of the pistillate parent from the pollen source. In this study, where seed recipients were detasseled and of the same variety as the pollen parent, distance was a significant factor ($P<0.001$) in seed set. The presence of high walls around the experimental plot could have influenced the wind speed within the test area; wind velocity measurements were not given. Still earlier studies examining outcrossing, and not merely pollen movement, as a function of distance from pollen source have been reviewed by Jones and Brooks (1950). Their own work indicated that an outcrossing rate of more than two per cent could occur at 40 rods (660 feet) from the pollen source:

The Percentage of Outcrossing Seed Occurring at Eight Distances of Isolation for Three Years.

	Distance north of contaminating fields (rods):							
Year	0	5	15	25	40	60	80	100
1947	35.18	16.48	5.13	0.82	0.44	0.15	0.15	0.15
1948	17.88	6.99	3.64	2.48	0.66	0.32	0.21	0.12
1949	32.87	19.17	8.60	3.68	2.47	0.99	0.32	0.32
AV	28.62	14.21	5.79	2.33	1.19	0.48	0.23	0.20

The authors attributed the yearly differences in outcrossing to seasonal variation: rainy weather and low wind velocity occurred during the pollinating season in 1948; hot, dry weather in 1947 could have contributed to the sharp decline in outcrossing beyond 15 rods in 1947. Each yearly data point in this study represents the harvest from 25 rows, each 3.5 feet apart, beginning at the

designated corn-free isolation distance. Looking at the outcrossing for 1949 produces the following:

Average Percentage of Crossing in 1949 of Yellow Suncropper onto Honey June for 25 Consecutive Rows at Eight Distances North of the Yellow Surecropper Field

	Distance north of contaminating fields (rods):							
ROW	0	5	15	25	40	60	80	100
1	90.62	80.40	28.07	4.33	6.28	2.98	0.66	1.42
2	77.62	46.18	13.64	12.48	0.66	0.32	0.21	0.12
3	39.92	44.07	40.07	8.30	7.91	3.45	0.43	0.39
4	54.83	36.17	33.54	5.17	6.79	2.34	0.00	0.27
5	46.11	27.94	1.88	3.62	0.68	0.71	0.08	0.90
6	31.89	35.23	17.12	4.89	3.61	2.05	1.31	0.33
7	43.37	11.96	9.33	2.35	1.77	1.01	0.26	0.13
8	45.74	8.36	11.80	1.83	1.56	1.02	0.04	0.17
9	28.25	16.16	5.81	1.72	0.42	0.00	0.10	0.00
10	26.96	20.23	1.72	6.96	0.75	0.00	0.52	0.08
11	29.49	14.26	4.55	2.86	1.28	0.03	1.42	0.00
12	32.88	21.52	3.18	0.59	1.15	0.65	0.04	0.68
13	25.05	9.88	2.16	1.72	2.21	1.51	0.00	0.06
14	26.37	13.40	4.94	1.30	1.07	0.54	0.30	0.65
15	23.20	7.38	7.42	1.90	1.31	0.39	0.10.	0.14
16	31.59	12.64	7.12	0.88	1.76	0.04	0.10	0.03
17	21.57	4.92	0.52	2.94	0.99	0.00	0.00	0.08
18	20.53	10.69	3.07	2.79	1.19	0.22	0.00	0.33
19	23.97	15.53	1.37	1.76	0.50	0.98	0.21	0.00
20	11.57	7.09	0.65	3.36	1.05	2.00	0.15	0.23
21	18.23	5.13	1.85	1.56	1.30	0.14	0.00	0.30
22	15.13	5.80	3.55	3.06	1.24	0.36	0.14	0.22
23	11.39	6.37	2.21	1.84	0.97	0.05	0.18	0.34
24	13.64	11.42	4.30	3.40	1.32	0.22	1.17	0.07
25	----*	10.40	5.07	---*	0.79	0.33	---*	0.05
Av.	32.87	19.17	8.60	3.68	2.47	0.99	0.32	0.32

*Blanks indicate data were not obtained

These data show a pronounced reduction (50%) in gene flow in the first five rows of the border rows of the pollen source and the decline, with isolation distance, of outcrossing at the first row of target plants.

The relatively short life of pollen also limits the effective distance pollen can travel. Under conditions of high temperature (Herrero and Johnson 1980) and desiccation (Hoekstra *et al* 1989), corn pollen longevity is measured in minutes; these conditions may even blast the tassel before any viable pollen is shed (Lonnquist and Jugenheimer 1943). More moderate conditions can extend the field life to hours (Jones and Newell 1948). Under laboratory

4. Depending on the experiment being conducted, the applicant has proposed several strategies to limit the possibility of volunteer transgenic corn being produced after the termination of field tests. These measures include: destroying transgenic plants before seeds are mature; hand harvesting of mature ears; monitoring during at least a 30 day fallow period following harvest; monitoring for volunteers the following growing season.

EFGWB believes that these measures will limit the possibility of volunteer transgenic corn persisting at the test sites.

5. The gene efficacy, insect susceptibility and plant breeding experiments as designed for the period April 1, 1993 through March 31, 1994 can **prevent** any transgenic pollen movement from the test sites to nontransgenic corn.

6. The resistance management and seed increase/hybrid production experiments for the period April 1, 1993 through March 31, 1994 are designed to **limit** transgenic pollen movement from the test sites to nontransgenic corn. With the proposed and recommended protocols, it is expected that outcrossing to corn outside the test sites can be limited to one per cent or less.

Chemical Exposure

1. Based on the data submitted and a review of the scientific literature, it appears that there is no unreasonable risk of offsite exposure to the CryIA(b) protein for the proposed field tests if pollen is prevented from maturing or is enclosed in protective coverings except during controlled pollen transfer to silks.

2. Based on the data submitted and a review of the scientific literature, it appears that proposed open-pollinated field tests of transgenic corn, using the containment measures recommended (See Biological Fate), can reduce, but not eliminate, offsite exposure to the CryIA(b) gene. With these measures, it is expected offsite expression of the cryIA(b) by pollen transport and gene capture through outcrossing can be reduced to less than one per cent. This assessment applies **only** for field tests to be completed on or before March 31, 1994. Insufficient information is available to make a chemical exposure assessment for the field tests

proposed to begin on or after April 1, 1994.

8. **RECOMMENDATIONS:**

Biological Fate

EFGWB recommends that the applicant provide the following additional information concerning the proposed field tests:

1. The minimum isolation distance from any other corn plants for the proposed insect susceptibility study at Research Park Triangle Park, North Carolina. The application merely states "No concerns for pollen escape exist for this location, as there are no other corn plants in the vicinity..."
2. The expected maximum period of receptive pistil exposure for nontransgenic corn plants in proximal fields temporally isolated from the proposed field trials. Given the diverse lines being tested, and possible differential degree day effects, how will temporal shifts in planting guarantee a poor nick, and a consequent limit to pollen exposure to receptive pistils of offsite, nontransgenic corn?

EFGWB recommends that the applicant make the following changes in the proposed experimental protocols:

1. Employ a 15 foot border of nontransgenic corn **and** an isolation distance of at least 660 feet from other corn for the resistance management and seed increase/hybrid production sites.

2. The applicant should also consider temporal shifts in planting for the breeding/seed increase sites as an additional, but not sole, limit to pollen movement to non-target corn (Schoultz 1985). The efficacy of this shift in producing an effective barrier to gene flow could be documented during the conduct of the 1993-94 trials.

Chemical Exposure

EFGWB recommends that the applicant provide the following additional information concerning the expression levels for the CryIA(b) and marker gene proteins in the transgenic corn:

1. Specific details of method validation pertaining to tissue preparation to determine whether or not the integrity of assayed proteins was maintained during processing;
2. Recovery efficiencies for target proteins and spikes, if any, assayed;

3. Expression levels of the transgenic proteins expressed on a per acre basis (grams/acre) in mature corn plants (maximum biomass) using the highest levels reported for each vector examined at the plant density indicated (i.e. 25,000 plants/acre) for these tests;
4. The percentage of the proposed field tests devoted to corn transformed with tissue specific (pollen and PEPC) or constitutive (CaMV) promoters.

EFGWB also recommends that, during the conduct of the 1993-94 field tests, expression levels of the transgenic proteins be determined for selected lines of field grown plants at points throughout the growing season to monitor changes in transgenic plant pesticide expression as a function of plant maturity. EFGWB suggests lines selected for these determinations reflect the range of expression expected; assays include tissues already reported (leaf, root, pith, pollen and fruit); and harvest intervals include transplant, pollen shed, fruit ripening and senescence stages of growth.

EFGWB has insufficient information from the applicant to make recommendations concerning chemical exposure for field tests proposed to commence on or after April 1, 1994.

9. **BACKGROUND:** NA

10. **DISCUSSION OF INDIVIDUAL STUDIES:**

Objectives of the experiment.

Gene efficacy evaluation: To evaluate the efficacy of CryIA (b), *B.t.* protein-producing corn hybrids for control of European Corn Borer (ECB) infestation and to determine the benefits conferred by such control.

Resistance management experiment: To determine how ECB respond to mixtures of corn plants when some of the plants within the mixture produce the CryIA (b), *B.t.* toxin.

Insect susceptibility study: To assess the field performance of CryIA(b), *B.t.* protein-producing corn plants against various lepidopteran pests and to study the interaction of the plants with various insect species, including beneficial predators and parasites.

Breeding: To introgress the cryIA(b) gene into elite corn lines and to make test crosses for future evaluation.

Seed increase/hybrid production: To increase seed of transgenic inbreds for use in hybrid production or feeding/safety studies on experimental animals and to produce hybrids with at least one CryIA(b), *B.t.* protein-producing parent for future field evaluations or feeding/safety studies.

Yield evaluations: To test pre-commercial hybrids for yield and other traits.

Population dynamics: To compare nontarget insect populations in fields of CryIA(b), *B.t.* protein-producing and control corn plants.

Test site locations.

Based on the data sunbmitted by the applicant, the following points are applicable to this EUP.

1. Transgenic plants of corn (*Zea mays* L.) expressing a CryIA(b) insect control protein derived from *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*) are proposed for field testing on 32.5 acres (104.7 acres including nontransgenic controls, border rows and alleyways) in nine locations in six states for the period ending on or before March 31, 1994.

2. Proposed field tests of corn containing the *B.t.k.* gene for the period April 1994 through March 1995 include 104.1 acres of transgenic plants at 24 sites in 17 states. Details concerning experimental protocols were not submitted for these tests. Consequently, total acreage (including nontransgenic controls, border rows and alleyways) could not be determined for tests proposed to commence on or after April 1, 1994.

PROPOSED SITES FOR PLANTING THROUGH MARCH 1994

State	County	Experiment(s)	Transgenic Acres	Total Acres
Florida	Palm Beach	Breeding	5.2	20.0
		Seed Increase	4.0	4.0
Hawaii	Maui	Breeding	7.8	30.0
		Seed Increase	12.0	12.0
Illinois	McClean	Gene Efficacy	0.6	11.0
		Breeding	1.3	5.0
	Shelby	Gene Efficacy	0.3	5.5
Iowa	Linn	Gene Efficacy	0.3	5.5
	Madison	Gene Efficacy	0.3	5.5
Nebraska	Seward	Gene Efficacy	0.3	5.5

North Carolina	Durham	Insect Susceptibility	0.1	0.2
	Johnson	Resistance Management	0.3	0.5
			32.5	104.7

PROPOSED SITES FOR PLANTING FROM APRIL 1994 THROUGH MARCH 1995

State	County	Experiment(s)	Transgenic Acres*
Florida	Palm Beach	Breeding	5.2
		Seed Increase	4.0
Hawaii	Maui	Breeding	7.8
		Seed Increase	24.0
Illinois	McDonough	Gene Efficacy	0.3
		Yield	0.3
	McClean	Gene Efficacy	0.3
		Breeding	1.3
		Seed Increase	12.0
		Yield	0.3
		Population Dynamics	2.1
	Shelby	Gene Efficacy	0.3
		Yield	0.3
	Champaign	Population Dynamics	2.0
		Gene Efficacy	0.3
	Ogle	Gene Efficacy	0.3
		Yield	0.3
	Lee	Seed Increase	12.0
	Sangamon	Seed Increase	12.0
Indiana	Wells	Gene Efficacy	0.3
		Yield	0.3
	Tippecanoe	Gene Efficacy	0.3
		Population Dynamics	2.1
	Madison	Gene Efficacy	0.3
Iowa	Linn	Gene Efficacy	0.3
		Yield	0.3

	Madison	Gene Efficacy	0.3
		Yield	0.3
	Kossuth	Gene Efficacy	0.3
		Yield	0.3
	Boone	Population Dynamics	2.1
		Gene Efficacy	0.3
Minnesota	Steele	Gene Efficacy	0.3
		Yield	0.3
	Waseca	Gene Efficacy	0.3
		Population Dynamics	2.1
Nebraska	Seward	Gene Efficacy	0.3
		Yield	0.3
	Lancaster	Gene Efficacy	0.3
		Population Dynamics	2.1
North Carolina	Durham	Insect Susceptibility	0.1
	Johnson	Resistance Management	1.0
		Population Dynamics	2.0
Wisconsin	Columbia	Gene Efficacy	0.3
		Yield	0.3
	Dane	Gene Efficacy	0.3
		Yield	2.1
			104.1

*Detailed experimental protocols and precise locations were not provided by the applicant. Total acreage (including nontransgenic corn and alleyways) could not be determined.

Strains and genotypes used.

Constructs will vary according to treatments and sites, but may include transgenic plants homozygous or hemizygous for the cryIA(b) gene as well as non-transgenic inbreds and hybrids (including commercial check cultivars). Transgenic plants were derived from transformation events 171 or 176. Plants from event 171 contain the synthetic cryIA(b) gene, the selectable marker *bar* (which codes for the enzyme phosphinothricin acetyltransferase, PAT, and confers resistance to phosphinothricin), and the scorable marker GUS (which codes for the enzyme beta-glucuronidase). These three genes are under the control of a CaMV 35S promoter. Plants

from event 176 contain the same synthetic cryIA(b) gene under control of two tissue-specific promoters: the phosphoenolpyruvate carboxylase (PEPC) promoter from corn and a pollen-specific promoter from corn. Event 176 plants also contain the *bar* gene under the control of a 35S promoter as a selectable marker (PAT) for phosphinothricin resistance.

Based on the data submitted by the applicant, the following points apply to this EUP.

1. The maximum CryIA(b) protein in seed planted through March 1994 is expected to be 35.7 grams.

2. The maximum CryIA(b) protein in seed planted from April 1994 through March 1995 is expected to be 112.0 grams. Details concerning experimental protocols were not submitted for these tests. Consequently, the possible increase in total CryIA(b) protein in seed planted from April 1994 through March 1995 from all anticipated sequential plantings could not be calculated.

3. Expression levels for the marker genes were not provided by the applicant for any plant tissue.

4. It appears that on-site exposure to the CryIA(b) protein may occur in roots, pith, leaves, pollen and seeds. Expression levels for the *B.t.k.* protein will vary according to the cassette inserted, plant transformed, tissue, stage of development and growing conditions at each test site. Levels reported by the applicant are listed in Table 1 of the Appendix.

5. The highest reported level of the CryIA(b) protein in seeds tested was 47 ng/g fresh weight (18 ng/mg soluble protein) for plants transformed with the PEPC/pollen promoter and 712 ng/g fresh weight (274 ng/mg soluble protein) for plants transformed with the CaMV constitutive promoter.

Test plot size and experimental design.

EFGWB notes that test protocols are given <u>only</u> for those experiments to be initiated before April 1994.

<u>Gene efficacy evaluation</u>:

Test plot size:
Area planted in transgenic plants not to exceed 0.3 acres /site;
Total acres, including alleys and buffer corn, not to exceed 5.5 acres/site.
Experimental design:

A split plot will be used, with infestation treatments as whole plots and genotypes as subplots.

Replicates:

3 replicates of each treatment combination.

Treatments:

1. Application of first brood ECB larvae. No application of second brood larvae. Insecticide protection for natural second brood infestation as needed;
2. Application of second brood ECB larvae. No application of first brood larvae. Insecticide protection for natural first brood infestation as needed;
3. No application of ECB larvae. Insecticide protection for natural infestations needed throughout season;
4. No application of ECB larvae. No insecticide protection for corn borer.

Plot design:

Each plot will consist of four rows, spaced 30 inches apart. Each row will be 17-21 feet long and contain a maximum of 35 plants. Each plot will be surrounded by up to four rows of buffer corn to reduce the chance of infestation of corn borer larvae which may migrate from adjacent plots and to serve as pollen source.

Resistance management experiment:

Test plot size:

Area planted in transgenic plants not to exceed 0.3 acres /site;

Total area not to exceed 0.5 acres/site.

Experimental design:

"The experiment will be set out as a split plot design with the genotype of the unifested plants being the main plots. This will allow the corn to be planted with a mechanical planter. The center plant in each replicate will be planted by hand after the original seed in the center position is removed."

Replicates:

Not determined at this time (see Plot design).

Treatments:

Each treatment will consist of one ECB infested center plant flanked by 3 rows of uninfested plants on either side and approximately 3.5 meters of unifested plants within the same row as the infested plant. Two ECB egg masses will be used to infest each center plant. Possible plant genotypes include:

1. Center plant produces CryIA(b) protein, uninfested plants do not produce CryIA(b);
2. Center does not produce CryIA(b), uninfested plants do not produce CryIA(b);
3. Center plant does not produce CryIA(b), uninfested plants produce CryIA(b);

4. All plants produce CryIA(b) protein.

Plot design:

"The number of replicates..[and] number of rows and row lengths will be determined once the amount of CryIA(b)-expressing seed that will be available is known. Ideally, 10 replicates per treatment would be used. This would require 40 small plots, each composed of seven rows, each seven meters long. This would be approx. 1960 square meters (less than 0.5 acre). If quantities of CryIA(b)-expressing seed are limited, treatments 3 and 4 may be eliminated, and replication of treatments 1 and 2 increased.

Insect susceptibility study:

Test plot size:

Area planted in transgenic plants not to exceed 0.1 acre /site;

Total area not to exceed 0.2 acre/site.

Experimental design:

Not given. See treatments.

Replicates:

Not given.

Treatments:

"Prior to planting, segregating seed will be germinated in the greenhouse. Plants will be screened for insecticidal activity using a[n] ECB bioassay and possibly also by qualitative ELISA to determine the presence of CryIA(b) protein. Positive plants will be used in the insect studies, and negative plants (presumably negative segregants) will be used as controls. Plots of CryIA(b)-expressing and nonexpressing plants will be artificially infested with ECB, corn ear worm, southwestern corn borer, and fall armyworm. Controlled releases of known parasites and predators of ECB (e.g., ladybird beetles, parasitic wasps, and lacewings) will be made, and any secondary effects on these populations will be documented. Interaction of the plants with other biological control agents such as *Beauveria* and *Nosema* may also be evaluated."

Plot design:

The plot will contain up to 12 subplots, each approximately 10 feet X 30 feet; subplots will consist of five rows of 20 plants per row, bordered by two rows of nontransformed plants.

Breeding:

Test plot size:

Area planted in transgenic plants not to exceed 1.3 acres /site with no more than 35,000 plants;

Total area, including alleys and buffer corn, not to

exceed 5.0 acres per site.

Experimental design:

Not applicable.

Replicates:

Not applicable.

Treatments:

Transgenic plants may be homozygous for the CryIA(b), hemizygous for the gene, or may have lost the CryIA(b) gene through genetic segregation. Nontransgenic inbreds will be used as checks and/or sources for breeding work.

Plot design:

The test plot will contain up to 100 rows of transgenic plants and 1000 rows of standard inbreds. Each row will be up to 21 feet long, containing up to 35 plants. Rows will be spaced 30 inches apart. The entire experimental plot will be surrounded by a border of at least 15 feet of nontransgenic inbred or hybrid plants which may also be used to make controlled hand pollinations.

Seed increase/hybrid production:

Test plot size:

Seed increase: 4.0 acres in Florida; 12 acres in Hawaii.

Breeding: 5.2 acres in Florida; 7.8 acres in Hawaii.

Experimental design:

NA.

Replicates:

NA

Treatments:

Transgenic plants may be homozygous for the CryIA(b) gene, hemizygous for the gene, or may have lost the CryIA(b) gene through genetic segregation. Nontransgenic inbreds will be used as parents for hybrid crosses. Test plots for seed increases will be solid-planted with transgenic seed at the rate of 25,000 plants per acre and allowed to open pollinate.

Plot design:

Test plots for seed increases will be solid-planted with transgenic seed and allowed to open pollinate. Plots will be planted at the rate of up to 25,000 plants per acre.

Yield evaluations:

Protocols are not provided. These experiments are not scheduled to be initiated before April 1994.

Population dynamics:

Protocols are not provided. These experiments are not scheduled to be initiated before April 1994.

Inoculation sampling and methods.

EFGWB notes that test protocols are given only for those experiments to be initiated before April 1994.

Gene efficacy evaluation:

"During the growing season data will collected for stand, visible corn borer damage and stay-green. Tissue samples may be removed from the plot for laboratory analyses. At harvest, data will be collected from the center two rows of each plot for grain yield, test weight, moisture and stalk strength. Some stalks will be destructively sampled to determine tunneling due to corn borer. Other data may be collected as needed."

Resistance management experiment:

"The major component of this experiment will involve analysis of ECB behavioral response to corn plants containing CryIA(b) protein, but data on growth and survival will also be collected. The experimental design will involve one ECB infested, center plant, flanked by three rows of uninfested plants on either side, and approximately 3.5 meters of uninfested plants within the same row as the infested plant. Two ECB egg masses will be used to infest each center plant. Once larvae emerge from their egg masses, their behavior will be closely monitored to determine the rate at which larvae redistribute themselves on the center plant and disappear from the center plant."

Insect susceptibility study:

See Treatments.

Breeding:

"Plant tissue samples may be collected from the plots for laboratory analysis. Plots at the Illinois location may also be infested with ECB larvae to evaluate the activity of the cryIA(b) gene in the field."

Seed increase/hybrid production:

"Plant tissue samples may be collected from the plots for laboratory analysis."

Yield evaluations:

Protocols are not provided. These experiments are not scheduled to be initiated before April 1994.

Population dynamics:

Protocols are not provided. These experiments are not scheduled to be initiated before April 1994.

Field test duration.

EFGWB notes that test protocols are given only for those experiments to be initiated before April 1994.

Gene efficacy evaluation:

Planting: Between April 1 and June 15, 1993
Harvest: Between September 1 and November 1, 1993

Resistance management experiment:

Planting: Late March 1993
Harvest: Before seed maturity, possibly in late July

Insect susceptibility study:

Planting: On or about April 1, 1993.
Harvest: Up to five months (September) after planting

Breeding:

Up to four plantings will occur at the Florida site during the period between January, 1993 and March, 1994. Planting dates will be scheduled according to seed availability and needs.

Up to six plantings will occur at the Molokai, Hawaii site during the period between January, 1993 and March, 1994. Planting dates will be scheduled according to seed availability and needs.

Planting at the Illinois site will occur Between April 15 and June 30, 1993.

Harvest at all sites will occur 3.5 to 5 months after planting.

Seed increase/hybrid production:

No more than one planting will occur at the Florida site during the period between July, 1993 and March, 1994. Planting dates will be scheduled according to the availability of seed and need

Up to three plantings will occur at the Hawaii site during the period between July, 1993 and March, 1994. Planting dates will be scheduled according to availability fo seed and experimental needs.

Harvest at both sites will occur 3.5 to 5.0 months after planting.

Yield evaluations:

Not given.

Population dynamics:

Not given.

Test site sanitation and monitoring.

EFGWB notes that test protocols are given only for those experiments to be initiated before April 1994.

Gene efficacy evaluation

Seed planters will be monitored to insure that no seed remains in each unit after each plot has been planted; transgenic corn will be detasseled prior to pollen shed; transgenic plots will be hand harvested; transgenic seed not retained for further study will be made nonviable by a method such as grinding or incineration; plant residue will be returned to the experimental field and incorporated into the soil to decompose. Fields will not be monitored for volunteer corn the following season.

Resistance management experiment

Machine and hand planting will be used (see experimental design); test plots will be isolated from other corn by at least 660 feet; plants will be cut down and disced into the soil before seeds are mature ("possibly in late July"); the field will be monitored for the remainder of that growing season for volunteers. Fields will not be monitored for volunteers the following season.

Insect susceptibility study

Planting methods are not described; all corn in the experimental plots will be open pollinated; isolation will be used for containment ("No concerns for pollen escape exist for this test location, as there are no other corn plants in the vicinity; the plot is surrounded by either mixed pine forest or Ciba building structures."); seed not for research purposes will be destroyed; plant residue will be soil incorporated; the field will be monitored for the remainder of that growing season for volunteers. Fields will not be monitored for volunteers the following season.

Breeding

Plots will be established by machine planting or hand planting of seed or by tranplanting; machine planters will be monitored to insure that no seed remains after each plot has been planted; tassels of all transgenic plants will be covered with a weatherproof bag prior to pollen shed and remain covered, except for controlled pollen transfer to silks, until pollen shed is

complete; experimental plots will be surrounded by at least 15 feet of nontransgenic corn; the entire plot, including the border corn will be separated from other corn fields intended for market, commercial or hybrid seed production by a distance of at least 660 feet, or temporally isolated by being planted at least two weeks before or after any nearby production fields; any open-pollinated corn will be discarded at the site or harvested and destroyed by such methods as grinding or incineration; any residue will be returned to the experimental field and soil incorporated to decompose; the Florida and Hawaii sites will remain fallow and be monitored for volunteers for at least 30 days (during this time the field will be irrigated); at the Bloomington site the experimental plots will be planted with a crop other than corn the next season and monitored for any volunteer corn.

Seed increase/hybrid production

Plots will be established by machine planting or hand planting; machine planters will be monitored to insure that no seed remains after each plot has been planted; the experimental plots will be separated from other corn fields intended for market, commercial or hybrid seed production by a distance of at least 660 feet, or temporally isolated by being planted at least two weeks before or after any nearby production fields; ears will be hand or machine harvested; machines will be cleaned at the test plot to remove all seed; plant material remaining in the field will be soil incorporated to decompose; the experimental plot will remain fallow and monitored for volunteers for at least 30 days (during this time the field will be irrigated).

Yield evaluations

Protocols are not provided. These experiments are not scheduled to be initiated before April 1994.

Population dynamics

Protocols are not provided. These experiments are not scheduled to be initiated before April 1994.

According to the applicant: "Details of the experimental protocols and precise locations for proposed experimental activities for planting between April, 1994 and March, 1995] cannot be determined at the present time, because these will partly depend upon the results of earlier studies. Protocols, methods, and objectives for the first five types of studies...are expected to be similar to the activities outlined earlier for the plantings through March 1994....Additional details concerning study design, cooperators, and site locations will be provided as far in advance of the start of the studies as is feasible. It is anticipated that the same standard for gene containment will be achievable, although the methods may need to be modified to accommodate larger acreage.

(For example, hand harvesting of large plots will be impractical. Machine combining would be necessary; appropriate measure for inspecting and cleaning of machinery would be used to insure that seed kernels did not escape containment)."

11. **COMPLETION OF ONE-LINER**: NA

12. **CBI APPENDIX**: NA

Table 1

Quantification of Cry1A(b) protein levels in various tissues of maize. Values are ng Cry1A(b)/mg soluble protein ± standard deviation.

	LEAF		ROOT	PITH	POLLEN/ ANTHER	KERNEL
35S LINES						
CG00554 x 171-18	513±244	(n=4)	596	288	NT	53
CG00554 x 171-4A	1732±39	(n=2)	NT	NT	NT	NT
CG00554 x 171-13	767±842	(n=8)	1209	4381	0	274
CG00554 x 171-14A	655±554	(n=7)	3348	2440	0	49
CG00615 x 171-16BB	283±227	(n=4)	74	71	0	NT
PEPC\POLLEN LINES						
CG00661 x 176-10	1703±378	(n=9)	52	60	260	15
CG00554 x 176-11	1288±583	(n=12)	54	113	418	16
CG00642 x 176-10	1138±188	(n=4)	NT	NT	NT	NT
CG00689 x 176-11	1077±108	(n=3)	NT	NT	340	NT
176-11 x CG00526	1842±345	(n=2)	47	53	NT	18

Values for control plants analyzed by ELISA are 0 ng.
Plants from event 171 contain the chimeric CaMV 35S/Cry1A(b) gene while plants from event 176 contain the chimeric PEPC/Cry1A(b) and pollen-specific/Cry1A(b) genes.
NT=not tested.

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Bt DELTA ENDOTOXIN AS EXPRESSED IN CORN SH#006430

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Pages 33 through 46 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

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