1402 A

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

1PP

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

3/27/92

MEMORANDUM

SUBJECT: OPP Final Scientific Position of the November 15, 1991, Monsanto Company Application for an Experimental Use Permit for an Insecticidal Toxin Produced by a *Bacillus thuringiensis* Gene in Cotton Plants.

TO: Phillip O. Hutton, PM 18
Insecticide/Rodenticide Branch
Registration Division (H7505C)

FROM: William R. Schneider, Ph.D.
Biotechnology Coordinator
Science Integration and Policy Staff
Environmental Fate and Effects Division (H7507C)

THRU: Amy S. Rispin, Ph.D., Chief
Science Analysis and Coordination Staff
Environmental Fate and Effects Division (H7507C)

Reviewers:

Leo R. LaSota, Ph.D.	Biologist	Environmental Fate & Ground Water Branch
Tom McClintock, Ph.D.	Microbiologist	Health Effects Division
David C. Bays, Ph.D.	Plant Pathologist	Ecological Effects Branch

OPP Position Document:

William R. Schneider,Ph.D.	Microbiologist	Environmental Fate & Effects Division

SUMMARY:

This document discusses the Office of Pesticide Programs' (OPP) scientific position on Monsanto Company's 11/14/91 Application for an Experimental Use Permit (EUP), No. 524-EUP-TG, for field testing of six similar versions of an insecticidal toxin as produced from *Bacillus thuringiensis* var. *kurstaki* (B.t.k.) delta-endotoxin genes introduced by an appropriate genetic vector into cotton plants. The experimental program is designed to evaluate B.t.k. (strains HD-1 and HD-73) insect control proteins, both full length and truncated forms, from three different genes (cryIA(b), cryIA(c), and cryIIA) for efficacy, host plant resistance, populations dynamics and threshold treatment. In addition to the B.t.k.

1819

proteins, the cotton plants express the enzyme neomycin phosphotransferase II (NPTII) which is used as a selectable marker during the plant transformation process.

The application is for testing at twenty-three sites in ten states. The typical experiment will be performed on one acre sites, but the inclusion of 71 acres of breeding nurseries (including two 20 acre sites in Mississippi, and two 10 acre sites in Arizona) will bring the total EUP area to 96.0 acres. The total size including borders, alleyways and buffer areas could be 238 acres. The seeds used in this experiment will contain approximately 66 grams of the modified B.t.k. delta-endotoxin insect control protein. Since the program will be conducted on a crop-destruct basis, with only some of the vegetative material, seed and lint retained for future research and planting purposes, tolerances for the pesticide residue will not be necessary for this EUP. The Office of Pesticide Programs (OPP) has evaluated the data submitted by Monsanto and, based on this data and other available data, can foresee no significant risk to humans or to nontarget organisms from this group of field tests as proposed by Monsanto as long as OPP containment recommendations are followed.

II. BACKGROUND

1. EUP APPLICATION REVIEW PROCESS

Monsanto Company submitted this application to the Office of Pesticide Programs (OPP) for an Experimental Use Permit (EUP) on November 14, 1991. Similar Monsanto constructs have previously been tested in small scale field tests under permits granted by USDA Animal and Plant Inspection Service (APHIS). Under an agreement with APHIS, OPP had received these previous submissions for comment. OPP is currently considering whether these products should be regulated by EPA. In the meantime, Monsanto has submitted this EUP application on a voluntary basis. A Federal Register Notice of Receipt which solicited public comment was published January 13, 1992. The submission was reviewed by OPP reviewers in the Environmental Fate and Ground Water Branch, the Ecological Effects Branch, and the Health Effects Division. The individual reviews were integrated into this OPP Preliminary Scientific Position (January 28, 1992). It should be noted that some aspects of the genetic construction were identified as Confidential Business Information (CBI). This information, two of the amino acid sequences for the six varieties of the toxin proteins and some of the construct details for four of the seven vectors, is not essential to the risk assessment and is not discussed in this Scientific Position. The Preliminary Scientific Position was made available for public comment (Federal Register, Friday, January 31, 1992) and submitted to a subcommittee of the OPP Scientific Advisory Panel (SAP) on February 25, 1992. The SAP comments were incorporated into this final Scientific Position.

2319

2. PRODUCT ANALYSIS

a. Protein Products and Associated Genes:

Truncated *B. thuringiensis* var *kurstaki* HD-1 delta-endotoxin (vectors: PV-GHBK01, PV-GHBK07), truncated *B. thuringiensis* var *kurstaki* HD-73 delta-endotoxin (vector: PV-GHBK02), and four variations of full-length *B. thuringiensis* var *kurstaki* HD-73 delta-endotoxins (vectors: PV-GHBK04, PV-GHBK05, PV-GHBK06); the kanamycin resistance marker gene, neomycin phosphotransferase (NPT II), from a bacterial Tn5 transposon will be used in these tests.

b. Plants:

Transgenic and non transgenic plants of *Gossypium hirsutum* (cotton) will be used in these tests:

Transgenic: Homozygous and segregating progeny of Coker 312 containing the transgenic vectors and crosses therewith; isoline hybrids between B.t.k. cotton and isolines with host plant resistance (HPR) traits;

Non transgenic: Coker 312, Stoneville 213, TAMCOT Cam D-E, DP 61, breeding and research lines, adapted cultivars specific for each test location.

c. Construction of Transgenic Plants:

The B.t.k. delta-endotoxin genes were transferred into plants using seven different *Agrobacterium tumefaciens*-mediated plant transformation vectors of both single and double border types (attachment 1). These plasmid vectors are constructed to contain the genes to be transferred to the plant cells, including the desired delta-endotoxin gene, appropriate transcriptional and translational control sequences to allow expression of the introduced genes in the plant, and an NPTII marker gene to allow for selection of the transformed plant cells. The vectors are incorporated into one of two strains of the bacterium, *A. tumefaciens* which contains plasmids that facilitate transfer of the vectors. When the *A. tumefaciens* is co-incubated with the plant cells, the vectors are transferred into the cultured plant cells. (Wild-type, plant pathogenic, strains of *A. tumefaciens* use this Ti transfer plasmid to produce plant galls, but these disarmed strains lack the phytohormone genes and cannot produce disease.) The cultured plant cells are selected for the desired characteristics and are regenerated into cotton seedlings.

3. DESCRIPTION AND LOCATION OF PROPOSED TESTING PROGRAM

a. Plant strains and genotypes used.

Transgenic cotton plants containing either GHBK01 or GHBK02 will comprise 90-95% of the transgenic plants tested. See attachment 2 for specific test details.

b. Specific test descriptions.

Economic Threshold Evaluations

Objectives: To determine if and when insect infestation is high enough to cause economic damage to cotton containing the delta-endotoxin from B.t.k.

Methods: The plants will be assayed for damage and final yield. Data for these traits will be correlated with insect density to determine if and when insect pressure is high enough to cause yield losses in transgenic cotton.

Gene Evaluations

Objective: To screen new transgenic cotton lines under field conditions to identify lines with the best insect resistance.

Methods: Each line is evaluated in plots that have been sprayed weekly with an insecticide to control lepidopteran pests and in plots that have received no treatments. Comparing the differences in insect control between these treatments provides a method to compare the B.t.k. lines in the tests.

Host Plant Resistance Evaluations

Objective: To test potential synergy between the B.t.k. delta-endotoxin gene and known host plant insect-resistance traits in cotton.

Methods: These tests will evaluate lines with B.t.k. delta-endotoxin alone vs. those lines containing various host plant resistance traits. If favorable combinations are identified based on insect damage and yield data, the trait(s) could be added to B.t.k. delta-endotoxin-containing cultivars using conventional breeding.

Population Dynamics Studies

Objective: To determine the effects transgenic cotton will have on arthropod populations.

Methods: Based on the earlier Monsanto field tests, cotton producing the B.t.k. delta-endotoxin will control many lepidopteran insects. Beyond that, the B.t.k. delta-endotoxin could theoretically have effects on other insects in a cotton field. To evaluate these potential effects, the transgenic cotton will be planted in a single large plot with no insecticide application. Adjacent to this plot will be two plots of non-transgenic cotton, one sprayed for lepidopteran pests as needed and the other left untreated. Throughout the season, the plots will be monitored for the incidence and quantity of various insects. Data, both positive and negative, will help determine what effects the three treatments have on both damaging and beneficial insects in the field.

Threshold Treatment Determination

Objectives: To ascertain if and when pesticide applications may be needed to supplement the protection provided by B.t.k. delta-endotoxin.

Methods: In previous field tests, transgenic cotton has exhibited insect control at or near the level provided by insecticides. However, the transgenic cotton was compared to cotton that had been sprayed weekly with an insecticide, far more frequently than recommended. These tests are designed to compare the effectiveness of transgenic cotton to non-transgenic cotton that has been treated as needed, a more realistic agricultural situation. Additional treatments include insecticide applications if certain damage levels are observed in the transgenic cotton. These tests will provide a good comparison of the yield protection provided by transgenic cotton with and without additional insecticide treatments and by non-transgenic cotton treated as needed.

Breeding Nursery

Objective: To introduce the genetic constructs into other cotton cultivars.

Seed Increase

Objective: To increase seed quantities of B.t.k. delta-endotoxin lines for future testing purposes.

c. Location and size of test plots: (See attachment 3 for details.)

Monsanto submitted an amendment to the EUP application, March 11, 1992, deleting 0.5 acre Economic Threshold experiments in Arizona, Mississippi, and South Carolina. This table reflects these changes:

State	Number of Sites	Acreage
Alabama	1	1.0
Arizona	3	22.0
Arkansas	1	7.0
California	2	4.0
Georgia	1	1.5
Hawaii	1	2.0
Louisiana	2	2.0
Mississippi	4	46.5
North Carolina	2	1.0
Texas	6	9.0
TOTAL	23	96.0

5819

d: Containment and mitigation plans.

Physical isolation: Cotton is not wind-pollinated (attachment 5). Monsanto has stated that all experiments other than the Hawaii site will be surrounded by "up to 24 border rows" of non-transgenic cotton or separated by at least 0.25 miles from any other commercial cotton. This will decrease the chances of insect pollinators carrying transgenic pollen beyond the test area. The cotton in the border rows will be included with the experimental cotton for disposal.

At the Hawaii seed increase site, the experiment will be surrounded by 4 border rows of non-transgenic cotton to provide a trap for outgoing pollen carried by insects. No commercial cotton will be grown within 1/4 mile of the test. Previous surveys conducted in the area have located no *Gossypium tomentosun* (wild native cotton) within 1.5 miles of the site. One feral *Gossypium barbadense* plant (commercial cotton, growing wild) was found within 1/2 mile of the site. Seed collected from that one feral plant during the 1990/91 growing season exhibited no evidence of outcrossing from the earlier field tests conducted under APHIS permits.

In response to a request in the 1/28/92 OPP Scientific Position document that Monsanto must guarantee through physical barriers and/or other security measures that access to the test site will be limited to authorized personnel, Monsanto reported (letter, February 7, 1992, Serdy to Jaeger) that the site was isolated by sugarcane fields to the north and east, by a landfill and restricted military site to the west, and by the ocean to the south. They stated that "the cotton has been planted far into the field off the main road so it is very difficult for anyone to notice driving by". In addition, during the previous 3 years of seed increases at this site under APHIS permits, there were no known instances of unauthorized persons entering the plot area.

Weed control: Weed control will follow acceptable practices for cotton by using labelled herbicides and hand weeding.

Disease control: Diseases will be controlled by the use of labelled fungicides.

Insect control: Insects will be monitored for their appearance in the field. Insecticides labelled to control insect pests that are present will be used on an as needed basis, in accordance with labelled instructions and in a manner that is compatible with the objectives of all the experiments.

Harvest at all sites except Waimea, Hawaii: The cotton will be harvested by hand or with field plot harvesters. If a machine is used, it will be thoroughly cleaned to insure that no seed are taken off site by the machinery.

Harvest at Waimea, Hawaii: The open bolls will be hand picked at maturity up to five separate times. Harvested seed cotton will be placed in cloth bags that will not allow for seed escape.

Disposal and monitoring at all sites except Waimea, Hawaii: Some plant, seed or seed cotton samples will be sent to the Monsanto laboratories in Chesterfield, MO, or to Texas A & M University, College Station, TX, for further analysis. A portion of the seed will be processed (ginned, delinted, and treated) to generate seed for future field trials. Some seed will be retained by the cooperator at each breeding nursery site for future breeding, seed increases, or agronomic evaluations. After data collection and harvest, all of the remaining seed and seed cotton not saved or sent to Monsanto or to Texas A & M will be destroyed on the site by distribution in the field. The seed and remaining plant stubble will be mechanically incorporated into the soil. After the experiment is concluded, the test site will be monitored for two months for germinating seed which will be destroyed by plowing under or herbicide treatment.

Disposal and monitoring at Waimea, Hawaii: The bags containing the harvested seed cotton will be placed into locked metal dryer boxes in the back of pickup truck at the field at the time of harvest. The truck will be used to transport the seed in the boxes to the office/seed drying facility approximately 1 1/2 miles away. The boxes will be removed and placed on the dryer for approximately two to three days until the seed cotton is dry enough to be ginned.

At that point, the seed and the lint will be separated by ginning with a table-top gin and returned to a cloth bag. The lint and trash from the ginning process will be collected and burned to destroy any viable seed that might be present. The ginning process should take no more than three to five days. During that time, the seed will either be kept in the locked dryer boxes or in 55 gallon metal drums with the exception of the seed which is physically being ginned.

The drying/ginning facility is within a fenced compound which is kept locked during non-business hours. When all the seed cotton is ginned, the seed in the cloth bags will be placed into plastic bags that will be placed into 55 gallon drums where it will be treated with phosphine as specified in the USDA Plant Pest Quarantine treatment manual. The plastic mesh bags will be placed in cardboard boxes for shipment. The USDA-APHIS inspector at Kauai will make the final inspection and, upon the inspector's approval, will release the seed for shipment. The container will be shipped to St. Louis via air freight. The plant stubble and seed-containing trash from the harvest will be mechanically incorporated into the soil. For the two months following harvest, the field will be cultivated or sprayed with a herbicide as needed to eliminate any volunteer cotton plants.

III. RISK ASSESSMENT

1. Environmental Fate / Transfer of Genetic Material

a. Transfer to wild relatives: There are four species of cotton in the United States. Two of them, *Gossypium hirsutum* (upland cotton) and *Gossypium barbadense* (sea island cotton, pulpulu haole), are used commercially and escaped plants can be found growing in the wild in climates where they can survive the winter, i.e. southern Florida and Hawaii. In addition, two native wild species occur in the United States: *G. thurberi* and *G. tomentosum*. (See attachment 4.)

G. thurberi is found in southern Arizona in mountainous regions. The Casa Grande, Maricopa and Yuma, Arizona sites for this EUP are in desert valleys which provide distance and habitat isolation from populations of *G. thurberi*. Never-the-less, any gene exhange betweeen plants of *G. hirsutum* and *G. thurberi*, if it did occur, would result in triploid (3x=39), sterile plants because *G. hirsutum* is an allotetraploid (4x=52) and *G. thurberi* is a diploid (2x=26). Such sterile hybrids have been produced under controlled conditions, but they would not persist in the wild; in addition, fertile allohexaploids (6x=78) have not been reported in the wild (Stewart, 1991).

The only Hawaiian site requested for this EUP is for the seed increase nursery on the island of Kauai. Hawaiian cotton, *G. tomentosum*, has not been found growing wild on Kauai. Two surveys by Montgomery (1990, 1991) found no *G. tomentosum* growing -or reported growing- in the wild on Kauai, however, cultivated plants of *G. tomentosum* were reported as growing in a private garden 10 miles from the test site.

Upland, Hawaiian and sea island cotton are all tetraploids than can crossbreed (Beasley, J.O. 1940a,b, 1942). The tropical climate of Hawaii, which permits a true perennial habit for all three *Gossypium* species, poses a monitoring concern already experienced near the test site: "To reduce seed production and dispersal it [a plant of *G. barbadense* within the survey area] "had been chopped down in July, 1990 by this writer [Montgomery, 1991], but it has quickly regrown, and was flowering prolifically from Dec. to early March, 1991." Introgression has been claimed for what Stephens (1964) considered hybrid swarms of *G. barbadense* x *G. tomentosum*. The possibility of the capture and expression of the B.t.k. protein and NPTII enzyme by either species can be prevented by restricting pollen movement from the test site, denying unauthorized personnel access, destroying all propagules (seed, vegetative plant parts) not used for further study and monitoring for volunteers and suckers following harvest (See **Protocol Modifications** below). Assuming the adoption of these provisions to control capture and expression, we believe that the testing under this EUP will result in no significant unplanned pesticide production through expression of the B.t.k. delta-endotoxin or NPTII marker enzyme genes in wild relatives of the transformed cotton, *Gossypium hirsutum* L.

b. Transfer of the delta-endotoxin genes to feral cotton: The inability of plants or seeds of either of *G. hirsutum* or *G. barbadense* to survive freezing temperatures restricts their persistence as perennials or recurrent annuals to tropical areas. Feral populations of *G. barbadense* exist in parts of southern Florida (Percival, 1987), but feral populations of neither this species nor *G. hirsutum* have been reported near any of the continental test sites subject to this EUP. For the Hawaiian site, as noted earlier, one feral *Gossypium barbadense* plant was found within 1/2 mile of the site. Seed collected from that one feral plant during the 1990/91 growing season seed exhibited no outcrossing from the earlier field tests. The additional precautions (see **Protocol Modifications**) for prevention of the capture and expression of the *Bt* protein and NPTII enzyme by wild cotton will also serve to prevent gene transfer to feral cotton. Accordingly, we believe that the testing under this EUP will present no significant risk of unplanned pesticide production through expression of the B.t.k. delta-endotoxin or NPTII marker enzyme genes in feral populations of these species.

8819

c. Transfer of the delta-endotoxin genes to adjacent cultivated cotton: A containment strategy of 24 buffer rows of nontransgenic cotton, or an isolation distance of 0.25 miles from any other cotton, will minimize, but not eliminate, the possibility of capture and expression of the B.t.k. and NPTII genes by cultivated cotton growing near the test sites. Expected outcrossing rates of approximately 3% or less are expected in cotton adjacent to the last (24th) border row and significantly less in cotton isolated by a distance of 0.25 miles. (See attachment 5) This rate is the maximum expected and is considered to be an insignificant contribution to the total exposure for the purposes of the risk assessment.

2. Quantification of Protein Products in Cotton

a. Extraction efficiencies, spike and recovery efficiencies, and the expression levels for B.t.k. and NPTII in GHBK01 and GHBK02 are reported in attachment 6.

b. The expression levels of protein in cotton plants are summarized in Tables 1 and 2. The data was reported in terms of the following:

-The fresh weight of each tissue on a per plant basis .
-The amount of B.t.k. or NPTII protein, based on a gram fresh weight of the tissue.
-The amount of the respective protein on a per plant basis.
-The total amount of the respective protein contained in the whole plant.

c. In addition to the data in Tables 1 and 2, Monsanto reported that the level of the B.t.k. protein in pollen was below the limit of detection of (1) 0.3 μg per gram fresh weight for cotton containing the vector GHBK02, and (2) 0.2 μg per gram fresh weight for cotton containing the vector GHBK01.

REPORTED RESULTS:

Expression Levels of Protein in Cotton Plants

Table 1. Levels of B.t.k. HD-1 and NPTII proteins in tissues from PV-GHBK01.

	g fwt/plant	B.t.k. HD-1 µg/g fwt	B.t.k. HD-1 µg/plant	NPTII µg/fwt	NPTII µg/plant
Leaves	50	2.4	120	<0.09	<5
Stems	287	1.2	344	1.1	320
Roots	42	0.70	29	<0.14	<6
Seed	12	5.0	60	4.0	48
Bolls	215	0.84	180	<0.04	<9
			Total 733µg		Total 388µg

Serdy. 1991c. (p.5)

Table 2. Levels of B.t.k. HD-73 and NPTII proteins in tissues from PV-GHBK02.

	g fwt/plant	B.t.k. HD-73 µg/g fwt	B.t.k. HD-73 µg/plant	NPTII µg/fwt	NPTII µg/plant
Leaves	74	0.16	12	<0.14	<10
Stems	227	0.03	7	1.4	318
Roots	37	<0.002	<0.1	<0.02	<1
Seed	12	1.6	19	8.0	96
Bolls	130	0.06	8	<0.05	<7
			Total 46µg		Total 432µg

Serdy. 1991c. (p.5)

10819

2. Human Health

OPP does not foresee any human health risks/effects resulting from the proposed field tests for the following reasons: (1) There will be minimal human exposure; and (2) No human or mammalian health effects associated with the plant constructs have been identified and therefore no adverse effects in these populations are anticipated.

a. Human exposure: There is limited direct human exposure to the treated crop since 30 of 34 proposed test plots at the 23 test sites in the various states are under 10 acres, and there is no dietary exposure because, with the exception of some plant material (seed, lint) being held for future research and plantings, all treated crops will be destroyed at the termination of the study by tilling back into the soil and monitoring to ensure destruction of any subsequent volunteers or suckers. In addition, due to the stable incorporation of these genes into the plant genome horizontal movement of any of these genes is not expected to occur. As shown in the exposure assessment, above, this EUP is not likely to result in unplanned pesticide production through the expression of the Bt delta-endotoxin or NPTII marker enzyme genes. The per acre amount of the Bt delta-endotoxin and the NPTII marker enzyme, based on data submitted by the applicants, will be approximately 50 grams and 26 grams, respectively, for plants containing either of two of the seven constructs (PV-GHBK01, PV-GHBK02). The amount for the remaining constructs (PV-GHBK03 through PV-GHBK-7) calculated at a rate of 10 times that expressed in all tissues of PV-GHBK01 is not expected to exceed 439 grams of B.t.k. and 233 grams of NPTII per acre (attachment 6). Furthermore, due to the nature of the protein products, contained within the plant parts, OPP does not foresee significant human and/or mammalian exposure via the pulmonary, ocular, or dermal routes.

b. Health issues (see attachment 7): No potential human or mammalian health issues associated with the plant constructs are anticipated. In fact, the only new proteins synthesized in the transgenic plants are the NPTII (KAN resistance) and the B.t.k. insect control proteins. The bacterial spectinomycin / streptomycin (SPC/STR) resistance gene, which is integrated into the plant genome following transformation, is not recognized or expressed by the plant; consequently, the gene is nonfunctional.

3. Exposure and Effects on Nontarget Organisms

a. Nontarget Organisms: This EUP is for a limited duration and acreage. OPP estimates that minimal movement of the B.t.k. gene will occur during these field tests (3% or less). This should lead to a very low exposure to nontarget organisms. In addition, several microbial pesticides containing non-engineered B.t.k. proteins have been registered over the last few years and extensive avian and aquatic testing, and beneficial insect testing has failed to demonstrate any significant toxicity to these organisms. It is possible that the truncated forms of the toxin, used in three of the seven constructs, may affect a greater range of organisms than the the full-length forms identified in the microbial pesticides, but this is not a concern for this EUP due to the limited exposure.

There is a potential for weeds to be created by addition of traits that can give them a selective advantage. In this case, the B.t.k. delta-endotoxin could confer selective advantage

11819

(specific insect resistance) to cultivated cotton, but upland or sea island cotton has many additional constraints, e.g. hardiness, habit (shrub), reproductive (not asexually propagated), cultural (host to other pests not controlled by *B. thuringiensis*) and other limits, which have prevented them from becoming aggressive or weedy despite their long cultivation in the cotton-growing regions of the continental United States. Therefore, weedy or agressive characteristics in cultivated cotton grown for the EUP is not expected to be created or aggravated by expression of the B.t.k. delta-endotoxin or NPTII marker enzyme genes. In any event, this cotton will be destroyed at the conclusion of the field tests. In addition, the field tests are sufficiently well contained to prevent wild cotton from acquiring the B.t.k. delta-endotoxin and increasing any weedy characteristics.

b. Endangered Species Considerations: Since the field tests will be located in areas of the 11 states which do not have any known populations of endangered lepidopteran species, no risk to endangered insect species is expected as a result of the proposed field test. Based on the low exposure from the limited acreage and duration of the EUP, OPP feels that there will not be a situation for endangered mammals, birds, invertebrates, plants and aquatic species that warrants a formal review under the Endangered Species Act.

IV. PUBLIC COMMENT

A Federal Register Notice announcing receipt of this EUP application was published 12/13/91 and provided for a public comment period. In addition, a second public comment period allowing for comment on the Preliminary Scientific Position was announced in the Federal Register of 12/31/92. The application was placed in a public docket, control number OPP-50735. Public comments were received 1/13/91 from Dr. Edward Bruggemann, writing for the National Audubon Society and from Phillip C. Burnett, writing for the National Cotton Council of America. Dr. Bruggemann identified no risk concerns for these particular field tests. However, he expressed concern for risks that may be associated with commercial use and strongly encouraged EPA to develop a policy for regulating transgenic plants.

He stated that EPA should develop data requirements for registration that would allow assessment of the following concerns: (1) Although the normal form of delta-endotoxin produced by the bacterium appears to be non-toxic to humans, some of the Monsanto constructs produce a truncated form of the delta-endotoxin which has not specifically shown to be non-toxic to humans. If toxicity is seen, a tolerance may be required for dietary intake of cottonseed products. (2) Excessive use of delta-endotoxin in plants as well as in bacterial products is likely to induce insect resistance thereby decreasing the usefulness of this valuable pesticide. (3) If the plants become wild or if the gene is transferred to related wild species, weeds may be produced that are resistant to natural control by lepidopterans.

OPP agrees that pesticides produced by transgenic plants should be regulated and has been developing a policy to this end. Implementation of a regulatory system must be approached carefully since the production of transgenic pesticides is very different than that of chemical or other biological pesticides. In addition, their unique characteristics make it appropriate to consider the existing regulatory procedures. OPP has been devising a

12819

regulatory structure to address these differences and is close to finalizing a policy for public discussion. Data requirements for registration will be included as part of the policy.

The possibility that the truncated form of the delta-endotoxin may have some unique mammalian toxicity will be assessed before a food or commercial use is approved by OPP. In this case, the toxin may well be inactived by the human digestive system, or the toxin may be inactivated by processing of the cottonseed, thereby eliminating dietary exposure.

OPP agrees that insect resistance is of concern for large scale uses of this type of product. Conceivably the development of insect resistance could cause an adverse environmental effect if the resistant insects could escape whatever natural balance might exist between insects and B. thuringiensis in the environment.

Weediness and the potential disruption of an ecosystem balance is a concern for OPP and has been addressed in this risk assessment.

Mr. Burnett, writing for the National Cotton Council, testified in favor of early approval of the Monsanto EUP application. He pointed out that Monsanto has tested its cotton plants for the last three years in over 30 locations in seven states and the product has proved to completely control heavy populations of pests without insecticide use. This product allows for a considerable reduction in the total insecticide useage on cotton and will assist the management of synthetic pyrethroid resistant in cotton pests.

OPP recognizes the usefulness of this type of product and has given the review of this application a high priority. OPP is committed to the development and registration of safer pesticides as alternatives to more toxic and persistent conventional pesticides.

V. SCIENTIFIC ADVISORY PANEL REVIEW

A subcommittee of the Scientific Advisory Panel met on February 25, 1992, to consider the OPP Preliminary Scientific Position. They were asked to address specific issues (attachment 9) concerning risk factors for this EUP application. A Subpanel Report (attachment 10) was finalized March 9, 1992. For this EUP application, the Agency requested comments from the SAP with respect to the containment provisions, including the protocol modification recommended by OPP. The SAP agreed that the containment provisions will prevent the proliferation of delta-endotoxin in subsequent generations of cotton, except for the possibility of carryover of viable transformed seed in the soil through a mild winter in the more southern continental U.S. sites. They recommended a 12 month monitoring procedure following the test unless Monsanto could produce evidence that carryover at a site was not a reasonable concern. OPP has included this recommendation in the PROTOCOL MODIFICATIONS section of this Scientific Position document.

In addition, the Agency requested comments on commercial use issues, the SAP stated that the truncated forms of *Bacillus thuringiensis* delta-endotoxin would pose no unique risk to humans to other mammals nor would any unreasonable adverse effects be anticipated for nontarget environmental species. The Subpanel also stated that neither the delta-endotoxin produced in the cotton plant nor delta-endotoxin produced in a bacterial system

will be posttranslationally-modified. Therefore, the bacterially-generated product should be similar to the cotton-generated product and would thus be acceptable for purposes of generating sufficient test material for use in toxicology testing. They believed that the data on non-target species effects from the population dynamics study would be valuable, but may be of limited relevance to other cotton production areas. They developed recommendations to improve the usefulness of this type of study. Furthermore, the SAP urged Monsanto and EPA to actively engage in, and support empirical testing of pest resistance management strategies.

VI. CONCLUSIONS

1. Summary

Although the various toxins produced by *Bacillus thuringiensis* have been studied extensively, Monsanto is using a truncated form in three of its seven constructs. This may or may not allow for an increased host range. Pending further testing to fully evaluate any effect of the pesticidal toxin on human health and nontarget environmental species, OPP has evaluated the exposure potential to humans and nontargets from this particular EUP. The amount of toxin produced on the field test sites is not sufficient to cause concern. The potential for the toxin genes to be transfered by pollination via insect vectors to other plants outside the field site resulting in the production of additional amounts of toxin has been assessed. Any transgenic wild or feral cotton will not over-winter except in southern Florida and Hawaii. In any event, OPP believes that the containment procedures as described by Monsanto in this EUP application, and further updated in their letter of February 7, 1992, Serdy to Jaeger, and modified by OPP (**PROTOCOL MODIFICATIONS**, below), are adequate to prevent any significant pesticide production outside the test site.

1. Biological Fate (Attachment 8)

a. OPP believes that the testing under this EUP will not result in any significant unplanned pesticide production through the expression of the B.t.k. or NPTII marker enzyme genes in wild relatives of the transformed cotton, *Gossypium hirsutum* L.

b. The testing under this EUP is not likely to result in any significant unplanned pesticide production through the expression of the B.t.k. protein or NPTII marker enzyme genes in feral populations of *G. hirsutum* or *G. barbadense* in the continental United States.

c. The containment strategy of a minimum of 24 buffer rows of nontransgenic cotton, or minimum of an isolation distance of 0.25 miles from any other cotton, is expected to minimize, but not eliminate, the capture and expression of the B.t.k. protein or NPTII genes by cultivated cotton growing near the test sites. OPP estimates that the outcrossing rate at the last border row will be approximately 3% or less.

2. Chemical Exposure (Attachment 6)

a. The B.t.k. delta-endotoxin is expected to occur in roots, stems, leaves,bolls and seeds; The NPTII marker enzyme is expected to occur in stems and seed (See Tables 1 & 2, above). Neither protein was detected by the applicant in pollen, nectar or lint.

b. Amount, per acre, of the B.t.k. delta-endotoxin and the NPTII marker enzyme is expected to be approximately 50 grams and 23 grams respectively for plants containing GHBK01; 3 grams and 26 grams respectively for plants containing GHBK02. These constructs are contained in at least 90% of the transgenic plants to be tested.

c. Expression levels for the other constructs (GHBK03-GHBK07), were not quantified by validated ELISA, but were estimated by western blot analysis of leaf tissue for the B.t.k. delta-endotoxin only:

> "The level of B.t.k. in the leaf tissue of plants containing the PV-GHBK03 to PV-GHBK07 vectors ranges from levels similar to that estimated for plants containing PV-GHBK01 to levels up to five fold higher. If it were assumed that the levels of the B.t.k. protein were even up to 10 fold higher, in all tissues, than that for the plant containing PV-GHBK01, then the amount of B.t.k. protein in these would be approximately 7 mg per plant. Assuming 60,000 plants per acre, an acre would contain up to 420 grams... The level of NPTII in the plants containing these vectors was not determined directly, but is not expected to differ significantly from the first two that were described above as the promoter driving the NPTII gene is identical in all of these plants." Serdy, 1992
>
> According to the data submitted by the applicant, these constructs (GHBK03-07) are found in less than 10% of the transgenic plants being tested, and will be evaluated only at 6 test sites (gene evaluation or seed increase) with plants having GHBK01 or GHBK02 constructs and with nontransgenic lines.

e. The presence of B.t.k. delta-endotoxin, offsite, is expected to be limited to seeds resulting from outcrossing events with the transgenic plants.

f. Based on the data submitted, and an outcrossing rate of 3% beyond the buffer rows, a worst-case assumption for occurrence offsite is approximately 100 milligrams for the B.t.k. delta-endotoxin and approximately 200 milligrams for the NPTII marker enzyme (Based on levels submitted for GHBK01 B.t.k [5μg/g fresh weight] or GHBK02 NPTII [8μg/g fresh weight] X 1600 [pounds seed/acre] X 454 [grams/pound] X 0.03 [expected maximum rate of outcrossing]).

3. Health Effects.

OPP does not foresee any human health risks/effects resulting from the proposed field tests because there will be minimal human exposure. All significant toxin production is confined to the test site and these plants will not be used for food or feed purposes.

15819

4. Ecological Effects.

OPP forsees no significant environmental impact resulting from the limited acreage EUP since few non target species would be exposed and no endangered species are present in the vincinity of the tests.

The expression of the B.t.k. protein or NPTII marker enzyme genes in cotton grown for this EUP is expected to neither create nor aggravate any weedy or aggressive characteristics.

VII. PROTOCOL MODIFICATIONS:

1. All sites except Hawaii must have either a minimum of 24 non-transgenic bufffer rows of *Gossypium hirsutum* or be isolated from other cotton by at least 0.25 mile.

2. In order to prevent germination and subsequent regrowth of transgenic cottonseeds following termination of the field release, one of these three alternatives must be followed for all sites in the continental United States:

a. Produce evidence that carryover at a site is not a reasonable concern. An acceptable experimental procedure for demonstrating this is specified in Attachment 11, Memorandum, March 17, 1992, LaSota to Nelson, or;

b. For at least 12 months following the test, do not plant non-transformed cotton in the plots containing transformed cotton. Rather, the plots should be monitored during the following season to detect any volunteer cotton plants. The applicant should develop a rigorous monitoring protocol and submit findings to the EPA, or;

c. The plots may be replanted in the same or similar constructs of transformed cotton/B.t. if the field release is conducted under an appropriate regulatory permit.

3. At the Hawaiian site, since there will be no other cotton within 0.25 mile, 4 nontransgenic border rows may be utilized as suggested by Monsanto. It is not necessary to follow the full 12 month monitoring plan specified for the continental United States because delayed germination due to cold temperatures is not a factor for Hawaii; the postharvest temperatures are tropical. However, the following measures must be taken whether or not the site is to be planted in cotton during the 1993-1994 growing season:

a. Extend monitoring of test site for volunteers or suckers to 5 months following harvest; destroy any volunteers or suckers. Supplement natural rainfall with irrigation, if necessary, to stimulate seed germination. If no irrigation is available and the natural rainfall is insufficient to allow cotton seed germination, extend the monitoring to 12 months.

b. Resurvey area within 0.5 mile of the test site following harvest and destroy completely any feral plants of *Gossypium* spp.

16819

VIII. RECOMMENDATIONS FOR FURTHER TESTING TO SUPPORT REGISTRATION:

1. Because of reported variability of the ELISA techniques used in this study, OPP recommends continued work to determine and eliminate assay related (non-biological) variability.

2. To address tolerance issues, OPP recommends that the applicant determine the fate of the transgenic pesticide proteins during processing of cotton seed for oil, meal and other by-products.

3. An acute oral rodent study is recommended to address dietary toxicology. We recognize the importance of obtaining an appropriate test material for our recommended maximum hazard high dose protocols. Ideally, the pesticidal toxin(s) (the "pesticide product") should be obtained in relatively pure form and should conform as closely as possible to the active form of the toxin as produced by the plant. Feeding studies with "whole foods" would only be warranted as a last resort, when it has been sufficiently well-established that the pesticidal toxin cannot reasonably be obtained in sufficiently high quantities apart from the plant. If it is not possible to extract enough pesticidal toxin from the plant or to reasonably purify it from other materials in the extract, or if it is denatured in the process, the pesticidal toxin may be obtained prior to introduction of the pesticidal genes into the plant. One could probably obtain the pesticidal toxin from the inserted gene cassette upon synthesis in a bacterium. Proteins derived in this manner may need to be analyzed for significant differences between the same protein(s) produced in the plant.

4. Non target organism studies are recommended. The species selected will be dependent on the anticipated exposure to non targets due to the requested use patterns, and on the expected susceptibility based on experience with similar toxins. Support from population dynamics field testing will be helpful.

References

Beasley, J.O. 1940a. The origin of American tetraploid *Gossypium* species. Amer. Nat. 74: 285-286.

Beasley, J.O. 1940b. The production of polyploids in Gossypium. J. Hered. 31: 39-48.

Beasley, J.O. 1942. Meiotic chromosome behavior in species, species hybrids, haploids, and induced polyploids of *Gossypium*. Genetics 25-54.

Brown, H.B. and J.O. Ware. 1958. Cotton. Third edition. McGraw-Hill Book Company, Inc., New York.

Degener, O. n.d. Flora Hawaiiensis, or, New illustrated flora of the Hawaiian islands. [Family 221, Genus *Gossypium*, Species *tomentosum*] Otto Degener, Honolulu, HA. n.p.

Fryxell, P.A. 1979. The natural history of the cotton tribe. Texas A & M University Press, College Station, TX.

Green, J. M. and M. D. Jones. 1953. Isolation of cotton for seed increase. Agron. J. 45: 366-368.

Kareiva, P., R. Manasse and W. Morris. 1991. Using models to integrate data from field trials and estimate risks of gene escape and gene spread. *In* The biosafety results of field tests of genetically modified plants and microorganisms. Edited by D.R. MacKenzie and S. C. Henry. Agricultural Research Institute, Bethesda, MD. pp. 31-42.

Kearney, T.H. and R.H. Peebles. 1951. Arizona flora. University of California Press, Berkeley and Los Angeles.

McGregor, S.E. 1976. Insect pollination of cultivated crop plants. Agricultural Handbook No. 496. United States Department of Agriculture, Agricultural Research Service, Washington, D.C.

Meredith, W. R. , Jr. and R. R. Bridge. 1973. Natural crossing in cotton (*Gossypium hirsutum* L.) in the delta of Mississippi. Crop Sci. 13: 551-552.

Moffett, J.O. and L.S. Stith. 1972. Honey bees as pollinators of hybrid cotton. Environ. Entomol. 1: 368-370.

Moffett, J.O., L.S. Stith, C.C. Burkhart and C.W. Shipman. 1975. Honey bee visits to cotton flowers. Environ. Entomol. 4: 203-206.

Montgomery, S.L. 1990. *Gossypium* and pollinators at Kekaha, Kauai: a report to Monsanto Company of St. Louis, MO. In Serdy 1991b (op. cit.) n.p.

Montgomery, S.L. 1991. Cultivated and naturalized *Gossypium* and their pollinators at Kekaha, Kauai: a report to the Monsanto Company of St. Louis, MO. *In* Serdy 1991b (op. cit.). n.p.

Munro, J.M. 1987. Cotton. Second Edition. John Wiley & Sons, New York, NY.

Sappenfield, W. P. 1963. Estimates of natural crossing in upland cotton in southeast Missouri. Crop Sci. 3: 566.

Serdy, F. 1991a. Information submitted to the United States Environmental Protection Agency, Office of Pesticide Programs, Registration Division, in support of an application for an experimental use permit to ship and use a pesticide for experimental purposes only. EPA DP Barcode #: 171306.

18819

Serdy, F. 1991b. December 10, 1991 letter to Registration Division (H7505C), Office of Pesticide Programs, U.S. Environmental Protection Agency (EPA File # 524-EUP-TG) concerning "Response to questions on Monsanto's request for an experimental use permit to allow field testing of several forms of *Bacillus thuringiensis* var. *kurstaki* insect control protein as expressed in cotton."

Serdy, F. 1991c. December 23, 1991 letter to Registration Division (H7505C), Office of Pesticide Programs, U.S. Environmental Protection Agency (EPA File # 524-EUP-TG) concerning "Response to questions on Monsanto's request for an experimental use permit to allow field testing of several forms of *Bacillus thuringiensis* var. *kurstaki* insect control protein as expressed in cotton." [Data corrected and included under same cover as January 10, 1992 from Serdy to EPA (Serdy, F. 1992)]

Serdy, F. 1992. January 10, 1992 letter to Registration Division (H7505C), Office of Pesticide Programs, U.S. Environmental Protection Agency (EPA File # 524-EUP-TG) concerning "Response to questions on Monsanto's request for an experimental use permit to allow field testing of several forms of *Bacillus thuringiensis* var. *kurstaki* insect control protein as expressed in cotton."

Simpson, D.M. 1954. Natural cross-pollination in cotton. U.S. Dept. Agr. Tech. Bul. 1094.

Simpson, D. M. and E. N. Duncan. 1956. Cotton pollen dispersal by insects. Agron. J. 48: 305-308.

Stephens, S.G. 1964. Native Hawaiian cotton (*Gossypium tomentosum* Nutt.). Pacific Science 18: 385-398.

Stewart, J. M. 1991. Gene transfer between contiguous cultivated cotton and between cultivated cotton and wild relatives: report to Monsanto Company. *In* Serdy 1991a (op. cit.). pp 10-15.

Thies, S.A. 1953. Agents concerned with natural crossing of cotton in Oklahoma. Agron. j. 45: 481-484.