April 28, 1998

MEMORANDUM

SUBJECT: Transmittal of the Final Report of the FIFRA Scientific Advisory Panel Subpanel on *Bacillus thuringiensis (Bt)* Plant-Pesticides and Resistance Management, Meeting held on February 9 and 10, 1998

FROM: Elizabeth Milewski, Ph.D.
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TO: Lynn R. Goldman, M.D.
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The above mentioned meeting of the FIFRA Scientific Advisory Panel (SAP) Subpanel on *Bacillus thuringiensis (Bt)* Plant-Pesticides and Resistance Management was an open meeting held in Arlington, Virginia to review a set of scientific issues and specific questions posed by the Environmental Protection Agency (EPA) based on its "White Paper".

Please find attached the Subpanel's final report of findings discussed at the meeting.

Attachment: a/s

cc: Subpanel Members
Susan Wayland
Marcia Mulkey
Steve Johnson
Jeralene Green
Denise Kearnes
Donald Barnes
Vickie Ellis
Janet Andersen
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Report February 9 and 10, 1998

FIFRA SCIENTIFIC ADVISORY PANEL

Subpanel on *Bacillus thuringiensis (Bt)* Plant-Pesticides and Resistance Management

The Subpanel on *Bacillus thuringiensis* (*Bt*) Plant-Pesticides and Resistance Management of the FIFRA Scientific Advisory Panel has completed its review of the specific questions posed by the Environmental Protection Agency (EPA) based on its "White Paper" (EPA, 1998), which evaluated scientific data generated by companies in the 1996 growing season and submitted to EPA on plants expressing *Bt* delta endotoxins. The report also analyzed information gathered on the 1996 bollworm infestation in the Brazos Valley in Texas in 1996 and testimony offered at two public hearings held by EPA under Section 21 of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) on March 21, 1997, in Washington, DC and on May 21, 1997, in College Station, Texas.

The Subpanel discussions were conducted in an open public meeting held at EPA, Crystal Mall #2, 1921 Jefferson Davis Highway, Room 1126 in Arlington, Virginia on February 9 and 10, 1998. Public notice of the meeting was published in the Federal Register on January 14, 1998. The meeting was chaired by Dr. Ronald Kendall (Texas Tech U.). Other Subpanel members participating were Drs. Randy Allen (Texas Tech U.), Michael Caprio (Mississippi State U.), Tim Dennehy (U. of Arizona), Fred Gould (N. C. State U.), Dick Hardee (USDA-ARS), Richard Hellmich (USDA-ARS/Iowa State U.), Randall Higgins (Kansas State U.), William Hutchison (U. of Minnesota), Randall Luttrell (Mississippi State U.), Ken Ostlie (U. of Minnesota), Blair Siegfried (U. of Nebraska-Lincoln), Mark Whalon (Michigan State U.), and John Witkowski (U. Of Nebraska).

Oral and written statements were received from various groups: Jane Rissler (Union of Concerned Scientists), Alan Goldhammer (Biotechnology Industry Org.), Allan Noe (The American Crop Protection Assoc.), Frank Carter (National Cotton Council), Bobby Carson (cotton grower), Graham Head (Monsanto), Diane Shanahan (Mycogen Corp.), Ron Buatte (Snake River Ranch), Joseph Mendelsohn (Int'l Ctr. for Technology Assessment), Steve Diercks (Coloma Farm, Inc.), Jeff Stein (Novartis Seeds, Inc.), Tim Seifert (Seifert Farms), Rebecca Goldburg (Environmental Defense Fund), Paul Clark (Greenpeace), Sally Fox (Fox Fibre Inc.), Ludger Wess (consultant/molecular biologist), Larry Antilla (Arizona Cotton Growers), Dean Urmston (American Seed Trade Assoc.), and John H. Benedict (Texas A&M Univ.). Written statements, and other background documents, can be obtained from the EPA Docket Office, telephone number: 703-305-5805 (see Docket Number: OPP #00231). The EPA White Paper can also be obtained electronically from the EPA Home Page at: Federal Register--Environmental Documents--"Laws and Regulations" (http://www.epa.gov/fedrgstr/).

EPA provided the Subpanel with a list of 13 questions. These specific questions were considered by the Subpanel. Based on a review of all the documents presented by the Agency, the best knowledge of the Subpanel and the discussion at the meeting, the following analysis of the questions is unanimously presented as the Report of the Subpanel. The Subpanel's Report can also be obtained electronically at the address cited above

The Report, which follows, has been divided into the following sections:

I. CONSENSUS STATEMENT AND DEFINITIONS

Consensus Statement

The Subpanel agrees that the widespread use of crops that express *Bt* insecticides is in the public good by providing additional pest control options to producers and by reducing the use of conventional pesticides. The Subpanel also recognizes that the risks of the selection of strains of targeted insects with strong resistance to *Bt* toxins is real, and steps to mitigate these risks are also in the public interest. Therefore, the Subpanel strongly agrees with the need for resistance management programs designed to suppress the emergence of insect resistance to *Bt* toxins expressed in transgenic crop plants. The Subpanel also believes that the regulatory strategies used in development of such programs should provide growers with a sustainable approach and not discourage them from employing this very valuable and environmentally friendly technology.

These resistance management programs should be based on structured refuges designed to provide sufficient numbers of susceptible adult insects with a minimum of economic and logistical impact on producers. The Subpanel recognizes that the design of such optimal refuge strategies requires the use of computer models, which are useful tools for comparative analysis of refuge designs. Such models have made important contributions toward an understanding of resistance management, which resulted in the employment of a refuge/high-dose system to delay the development of resistance of Colorado potato beetle (*Leptinotarsa decemlineata* (Say))(CPB), European corn borer (*Ostrinia nubilalis* Hübner))(ECB), pink bollworm (*Pectinophora gossypiella* (Saunders))(PBW), and tobacco budworm (*Heliothis virescens* (Fabricius))(TBW) to *Bt* toxins expressed in transgenic crops. The Subpanel is in agreement that the precision of research models is limited by the unavailability of experimental data for many parameters, and thus, the accuracy of these models has not been tested under field conditions. Furthermore, the high degree of variability in the production systems now used for *Bt* expressing crops adds to the uncertainty. Therefore, the Subpanel feels that research models provide important information for evaluating refuge strategies, but models alone provide insufficient evidence for the establishment of specific refuge requirements. The Subpanel agrees that a refuge/high dose strategy must be employed for targeted pests within the current understanding of the technology. However, as pest biology and their response to *Bt* crops becomes better understood, there may be opportunities to reconsider and modify this strong recommendation. The Subpanel believes that regulatory strategies should serve to provide growers with a sustainable approach that encourages compliance for utilizing this valuable and environmentally friendly technology. Altered restrictions should be enacted at such time as definitive field data and/or resistance monitoring data demonstrate that less restrictive measures will not lead to resistance development within a long term planning horizon. In situations where recommended resistance management strategies are not economically and/or logistically feasible for growers to use, such management plans should be modified accordingly. The reason for this position is that grower participation is the key factor in successful implementation of resistance management, and grower acceptance is likely to hinge on economic and logistical feasibility. Thus to the extent possible, feasibility should figure in the development of resistance management plans. The needs of growers who rely on *Bt* sprays also should be taken into consideration when developing regulatory decisions for resistance management.

Therefore, the Subpanel recommends that the EPA require the use of structured refuges in all registrations of *Bt* crops unless it can be shown conclusively that such refuges would harm, rather than aid, durability of the resistance management plan. The Subpanel recognizes that regional differences in crop production practices and pest population parameters (including pest complexes) may require guidelines governing resistance management strategies to have some flexibility. Thus, acceptable refuge configurations may vary among regions. "Acceptability", in terms of resistance management, is based on the common premise that adequate numbers of unselected insects are produced in sufficient proximity to the *Bt* crops to ensure that resistant insects will rarely mate with each other.

Given the contextual nature of these requirements, the Subpanel recommends that the EPA establish working groups for each of the major *Bt* crop producing regions, and that these working groups include representatives from all stakeholders. These working groups will meet annually (or as deemed appropriate) and will be charged with the development of implementable *Bt* resistance management programs for their region that are based on the best available field data and model predictions. In addition, the working groups will identify regional research needs and coordinate remedial action plans. The Subpanel anticipates that these working groups would design context-specific resistance management programs that provide the necessary balance between reasonable assurances that *Bt* crop technology will remain viable in the long term while providing producers with practical and economically feasible refuge requirements.

Definitions

Definition of High Dose

The Subpanel discussed ways to define and measure "high dose" in plants. It was agreed that the definition of high dose as "25 times the toxin concentration needed to kill susceptible larvae" was reasonable based on current empirical data. However, the Subpanel recognized that it is conceivable that a heterozygote may develop with higher than 25-fold resistance.

The major problem identified by the Subpanel was in determining if this 25-fold level was achieved in a specified cultivar. After much discussion, it was concluded that there were at least 5 imperfect ways to assess this 25-fold level, and that some approaches were more appropriate for specific crop pests. The Subpanel concluded that a cultivar could be considered to provide a high dose if two of the five approaches described here indicated presence of a high dose.

The five approaches are:

(1) Serial dilution bioassay with artificial diet containing lyophilized tissues of *Bt* plants (tissue from non-*Bt* plants serving as controls);

(2) Bioassays using plant lines with expression levels approximately 25-fold lower than the commercial cultivar (determined by quantitative ELISA or some more reliable technique);

(3) Survey large numbers of commercial plants on sentinel plots in the field (e.g., sentinel sweet corn method) to make sure that the cultivar is at the LD99.99 or higher to assure that 95% of heterozygotes would probably be killed. With this approach *Bt* sweet corn hybrids are used to attract high densities of ECB and cotton bollworm (*Helicoverpa zea*)(Boddie)) (CBW/CEW) moths, sampling can be limited to sweet corn ears in the *Bt* plot (ca. 1/4-1/2 acre block), and a frequency of resistant phenotypes can be estimated as the ratio of density of larvae/plant in *Bt* sweet corn to density of larvae/plant in an adjacent planting of non-*Bt* sweet corn (Andow and Hutchison, 1998; Hutchison, unpublished data).

(4) Similar to (3) above, but would use controlled infestation with a laboratory strain of the pest that had an LD50 value similar to field strains;

(5) Determine if an older instar of the targeted pest could be found with an LD50 that was about 25-fold higher than that of the neonate larvae. If so, that stage could be tested on the crop plants to determine if 95% or more of the older stage larvae were killed.

Definition of Secondary Pest

To understand the concept of a secondary pest, it is necessary to define a primary pest as the "targeted" pest for which a *Bt* crop is planted. Thus, a secondary pest is a "non-targeted" pest which could be affected directly or indirectly in one of two ways when a *Bt* crop is planted to manage a targeted (primary) pest: (1) one that is directly or potentially affected by a low to high dose expressed in a *Bt* crop but is of less economic significance than the targeted pest, or (2) one that is indirectly affected by a *Bt* crop through changes in insecticide use patterns. An example of (2) would be cotton aphid (*Aphis gossypii* Glover) in *Bt* cotton which may be partially, or in some cases, consistently controlled by natural enemies (predators and parasitoids) because of reduced insecticide use. On the other hand, western bean cutworm (*Richia albicosta* (Smith)) on *Bt* corn in Nebraska and plant bug (Family Miridae) and stink bug (Family Pentatomidae) complexes in cotton, might become more prevalent in the absence of insecticides normally applied to non-*Bt* crops for the targeted pest. For each of the *Bt* crops, targeted and non-targeted pests within the two categories are listed by geographical region in the U.S. It is important to note that a non-targeted pest on one crop may actually be a targeted pest on another crop. This listing reflects what is currently understood about the activity spectra of registered *Bt* events. New events may have different activity spectra that could change how these or other insects are categorized.

<u>*Bt* Field Corn:</u>

(1) Targeted pests

European corn borer (*Ostrinia nubilalis* (Hübner)) (ECB)
southwestern corn borer (*Diatraea grandiosella* Dyar) (SWCB)

(2) Non-targeted pests -- directly or potentially affected by *Bt* crop (low to high *Bt* dose effect, dependent on corn event, toxin, and/or promoter)

common stalk borer (*Papaipema nebris* (Guenée))
corn earworm (*Helicoverpa zea* (Boddie))(CEW/CBW)
cutworms
- black cutworm (*Agrotis ipsilon* (Hufnagel))
- dingy cutworm (*Feltia jaculifera* (Guenée))
- other cutworms (Family Noctuidae)

fall armyworm (*Spodoptera frugiperda* (J. E. Smith))
hop vine borer (*Hydroecia immanis* Guenée)
potato stem borer (*Hydroecia micacea*) (Esper)
sod webworms (Subfamily Cramdinae)
true armyworm (*Pseudaletia unipuncta* (Haworth))

(3) Non-targeted pests -- indirectly affected by *Bt* crop through changes in insecticide use patterns

corn leaf aphid (*Rhopalosiphum maidis* (Fitch))
corn rootworm complex (*Diabrotica* spp.)
spider mites (*Tetranychus* spp.)
western bean cutworm (*Richia albicosta* (Smith))

Bt Cotton:

A. Mid-South and Southeast:

(1) Targeted pests

tobacco budworm (*Heliothis virescens* (Fabricius)) (TBW)
cotton bollworm (*Helicoverpa zea* (Boddie)) (CBW/CEW)

(2) Non-targeted pests -- directly or potentially affected by *Bt* crop (low to high *Bt* dose effect, dependent on cotton event, toxin, and/or promoter)

beet armyworm (*Spodoptera exigua* (Hübner))
cabbage looper (*Trichoplusia ni* (Hübner))
cotton leaf perforator *(Buccalatrix thurberiella* Busck) (very occasional)
European corn borer (*Ostrinia nubilalis* (Hübner)) (ECB - some locations, e.g., North Carolina)
fall armyworm (*Spodoptera frugiperda* (J. E. Smith))
southern armyworm (*Spodoptera eridania* (Stoll))
soybean looper (*Pseudoplusia includens* (Walker))

(3) Non-targeted pests -- indirectly affected by *Bt* crop through changes in insecticide use patterns

boll weevil (*Anthonomus grandis grandis* Boheman) (where it has not been eliminated)
cotton aphid (*Aphis gossypii* Glover)
cotton fleahopper (*Pseudatomoscelis seriatus* (Reuter))
cotton square borer (*Strymon melinus* Hübner)
spider mites (*Tetranychus* spp.)
stink bug complex
- brown stink bug (*Euschistus servus* (Say))
- green stink bug (*Acrosternum hilare* (Say))
- southern green stink bug (*Nezara viridula* (Linnaeus))

tarnished plant bug (*Lygus lineolaris* (Palisot de Beauvois))
whitefly complex
- bandedwinged whitefly (*Trialeurodes abutiloneus* (Haldeman))
- silverleaf whitefly (*Bemisia argentifolii* (Bellows & Perring))
- sweetpotato whitefly (*Bemisia tabaci* (Gennadius))

B. Southwest:

(1) Targeted pests

pink bollworm (*Pectinophora gossypiella* (Saunders)) (PBW - all areas of southwest)
cotton bollworm (*Helicoverpa zea* (Boddie)) (CBW/CEW - eastern New Mexico)
tobacco budworm (*Heliothis virescens* (Fabricius)) (TBW - eastern New Mexico)

(2) Non-targeted pests -- directly or potentially affected by *Bt* crop (low to high *Bt* dose effect, dependent on cotton event, toxin, and/or promoter)

beet armyworm (*Spodoptera exigua* (Hübner))
cabbage looper (*Trichoplusia ni* (Hübner))
cotton leaf perforator (*Buccalatrix thurberiella* Busck) (very occasional)
saltmarsh caterpillar (*Estigmene acrea* (Drury)
tobacco budworm (*Heliothis virescens* (Fabricius)) (TBW - western New Mexico, Arizona, California)

(3) Non-targeted pests -- indirectly affected by *Bt* crop through changes in insecticide use patterns

cotton aphid (*Aphis gossypii* Glover)
Lygus bug (*Lygus hesperus* Knight)
spider mites (*Tetranychus* spp.)
stink bug complex
- brown stink bug (*Euschistus servus* (Say))
- green stink bug (*Acrosternum hilare* (Say))
- southern green stink bug (*Nezara viridula* (Linnaeus))

whitefly complex
bandedwinged whitefly (*Trialeurodes abutiloneus* (Haldeman))
silverleaf whitefly (*Bemisia argentifolii* (Bellows & Perring))
sweetpotato whitefly (*Bemisia tabaci* (Gennadius))

Bt Potato:

(1) Targeted pest

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

(2) Non-targeted pests -- directly or potentially affected by *Bt* crop (low to high *Bt* dose effect, dependent on potato event, toxin, and/or promoter)

none

(3) Non-targeted pests -- indirectly affected by *Bt* crop through changes in insecticide use patterns

aphids (Family Aphididae)
aster leafhopper (*Macrosteles quadrilineatus* Forbes)
European corn borer (*Ostrinia nubilalis* (Hübner)) (ECB)
flea beetles (Family Chrysomelidae)
garden symphylan (*Scutigerella immaculata* (Newport))
grasshopper (Family Acrididae, primarily *Melanoplus* spp.)
leaf miners (*Liriomyza* spp.)
onion thrips (*Thrips tabaci* Lindeman)
potato leafhopper (*Empoasca fabae* (Harris))
potato psyllid (*Paratioza cockerelli* Sule)
potato tuberworm (*Phthorimaea operculella* (Zeller))
spider mites (*Tetranychus* spp.)
threelined potato beetle (*Lema trilinea* White)
wireworms (Family Elateridae)

Definition of Structured Refuges

Structured refuges include all suitable non-*Bt* host plants for a targeted pest that are planted and managed by people. These refuges could be planted to offer refuges at the same time when the *Bt* crops are available to the pests or at times when the *Bt* crops are not available.

Definition of Fitness Costs

Fitness costs are defined as the decrease in fitness of individuals with resistance alleles relative to individuals without resistance alleles, when both types of individuals are feeding on non-toxic plants.

The other type of fitness cost alluded to in this Report is the decrease in fitness of individuals on *Bt* plants relative to their fitness on non-*Bt* plants, even when those individuals have resistance alleles.

Definition of Halo Effect

The halo effect, relative to *Bt* corn and ECB, is defined as the suppression of ECB infestations in non-*Bt* corn blocks adjacent to a *Bt* corn field, or suppression in non-*Bt* corn strips within a *Bt* corn field, as a result of minimal moth emergence from the *Bt* corn (i.e., few survivors from first generation)(Andow and Hutchison, 1998).

II. RESPONSE TO QUESTIONS

Question 1: Pest Biology and Population Dynamics

A. The EPA issue paper has summarized what is known or current research regarding the biology and population dynamics, 1. Larval mobility and dispersal, 2. Larval feeding behavior (preferred plant feeding sites), 3. Mating behavior (adult movement, pre-mating behavior, flight range), and 4. Ovipositional behavior (host choice, egg deposition), of the key targeted pests of *Bt* potato (CPB), *Bt* corn (ECB, CEW/CBW, fall armyworm, SWCB), and *Bt* cotton (TBW, CBW/CEW, PBW). Is there further information needed for developing a long-term resistance management program in these areas?

There was a clear consensus among the Subpanel members that more research is necessary, especially regarding all the factors identified in this question. The broad-based discussion across all three *Bt* crops (potato, corn, cotton) centered around adult movement (pre- and post-mating dispersal), general mating behavior, ovipositional patterns, fitness and larval movement. Much of this discussion occurred in the context of refuge, its spatial and temporal dimensions, and its effectiveness in producing susceptible adults. Knowledge of adult movement between hosts, such as CEW/CBW between corn, cotton and wild hosts, is important to understanding overall population structure and contribution of weed and crop hosts to population size. Landscape ecology and impact of refuge management were identified as important issues that need to be addressed. Regional differences in factors such as field size, crop rotations, prospective crop use (e.g., chipping vs. table stock vs. seed for potato), and crop production practices were recognized as markedly impacting design of resistance management programs. The inheritance and frequency of resistance genes also play an important role.

While much research effort is directed at these issues, ongoing emphasis on these research topics is required for continual refinement and design of resistance management programs. Although research in areas identified in this question is primarily directed at targeted pest(s), it is important to remember that significant data gaps exist for non-targeted pests in these cropping systems. The importance of these non-targeted pests, however, will vary with the crop and geographical area. However, it is safe to say that the population dynamics of the various pests impacted by *Bt* crops are complex.

B. What pest biology information is most important to the formulation of long-term resistance management strategies (in particular, but not limited to the development of a refuge strategy) and how can it best be used to develop a long-term resistance management strategy?

This question is adequately discussed in the response to Question 1A above.

C. Discuss the role of secondary pests in resistance management strategies:

1. What defines a secondary pest? (relative economic damage, sporadic appearance and/or locality effects?)

The Subpanel provided a definition of "secondary pest" in Part I of this Report. *Bt* corn, *Bt* potato, and *Bt* cotton were recognized to have two possible effects on non-targeted pests. Non-targeted pests are either directly or potentially affected by the *Bt* toxin or indirectly affected by changes in *Bt* crop management, e.g., insecticide use that accompany control of the primary targeted pests. Non-targeted pests were categorized by these likely effects based on a current understanding of the activity spectra of *Bt* events (see Part I of this Report). Note: This listing reflects what is currently understood about the activity spectra of registered *Bt* events. New events that may have different activity spectra or expression levels could change how these or other insects are categorized.

The non-targeted pest complex differs among *Bt* crops and varies geographically. For example, corn has only a few non-targeted pests directly affected by *Bt* and these generally have restricted geographical importance (see Definition of Secondary Pest in Part I of this Report). With minimal use of foliar insecticide across most of the central corn belt, indirect effects on non-targeted pests from altered insecticide use patterns are not anticipated. The exception may be in western irrigated corn production, where insecticide use against ECB or SWCB may have incidentally suppressed some non-targeted pests, e.g., western bean cutworm, or induced outbreaks of other secondary pests, e.g., spider mites. Because of extensive insecticide use against the primary targeted pest of *Bt* potato, the CPB, non-targeted pests in potato are much more likely to be affected by changing insecticide use patterns.

In contrast, cotton has many targeted and non-targeted pests. The production system is dynamic, and outbreaks of non-targeted pests are commonly associated with changes in insecticide use patterns against primary pests, or indirect influences of other pest and non-pest dynamics. Some of these non-targeted pests, such as the soybean looper, are directly affected by *Bt* cotton and may be prone to resistance development. Resistance problems may be more severe for polyphagous pests exposed to *Bt* toxins in other crops. Other non-targeted pests, like stinkbugs and aphids, that are not directly affected by *Bt* cotton may be affected by changes in insecticide use patterns.

2. What are the resistance management considerations for secondary pests not controlled by the toxin?

Elimination or reduction in insecticidal sprays will have positive or negative effects on other non-targeted pests. Problems with insecticide-induced pests, such as the cotton aphid in cotton or spider mites in corn, will likely diminish as insecticide use against the primary target decreases. In contrast, other non-targeted pests incidentally controlled by insecticide applications targeted at the primary pest, may become more common and require their own distinct management efforts. Examples include: in *Bt* cotton - stink bugs and various plant bugs; in *Bt* potato - potato leafhopper and aphids; and in *Bt* corn - western bean cutworm in irrigated areas.

The aggregate impacts of *Bt* crops on IPM and resistance management of non-targeted pests are unknown at this time. Changes in the structure of cropping systems and the reduction in insecticide use will likely alter the pest complex and its threat to production. In turn, these changes will require flexibility and adjustment in pest management. Non-targeted pests in cotton, potato and corn will demonstrate varying susceptibility to *Bt* toxins. Despite efforts to present a high dose against the targeted pests, non-targeted pests are likely to demonstrate varying susceptibility to the toxins expressed by a *Bt* event. Low to moderate doses experienced by some non-targeted pests may need to be considered in resistance management strategies.

Question 2: Target Pest Dosage

A. Discuss the level of expression that constitutes a "high dose" of toxin in a plant-pesticide. In addition, discuss "moderate dose" and "low dose".

High dose:

A preliminary suggestion from the Subpanel was that a "high dose" results when a plant consistently produces enough toxin to kill all or most heterozygotes (i.e., individuals carrying one major resistance allele). The problem is that there may be types of heterozygotes that have not yet been found. It is, therefore, impossible to predict exactly what toxin level will kill each potential heterozygote genotype. This presents a dilemma. If a concrete definition of a high dose can not be offered, it will be difficult to develop regulatory strategies.

An operational definition from the literature (Gould *et al*., 1994) was offered as a potential approach for circumventing the dilemma. The definition states that a high dose results from a concentration of toxin that is at least 25-fold higher than the LD99 of susceptible strains of the pest species. This definition was developed based on examining the empirical literature on resistance levels in F1 offspring from crosses of *Bt* resistant and *Bt* susceptible strains of targeted and model insect species. From this literature it was found that no F1 larvae had more than 25-fold resistance compared to the susceptible strains.

Concern was expressed that for many insects it would be difficult to ascertain if plants were indeed producing this target concentration. Furthermore, it was argued that only limited empirical data on heterozygote resistance levels are available and that it was possible that some resistance alleles would be dominant and confer 100-fold or more levels of resistance. In such cases, a plant with 25 times the toxin level needed to kill susceptible larvae would not serve as a high dose.

A suggestion was offered that it would be better to assess high dose by direct observation of a cultivar's field performance. If the cultivar offered near or absolute protection against the targeted pest(s), that would be evidence it was expressing a high dose. The rationale behind this approach is that if the cultivar has a toxin level above the LD99.99, then there would be high mortality of heterozygotes.

Some Subpanel members expressed concern that the concentration of toxin in a plant could vary over the season and in response to environmental stress. Additionally, there is evidence that in some cultivars the toxin concentration varies among plant parts. Therefore, in determining "high dose" status, we must make sure we are truly measuring the toxin concentration encountered by the pest. A final comment was that in cases where the pest can move from non-*Bt* plants to *Bt* plants as the pest matures, there is a need to consider the LD99 level of older larvae which is typically higher than that of neonates.

Given these considerations, the Subpanel developed the operational definition of high dose found in Part I of this Report.

Moderate dose:

A moderate dose is expressed in a cultivar with a toxin concentration that is below the high dose for a given insect but initially offers economically acceptable control as a stand-alone pest control tool.

Low dose:

A low dose is expressed in a cultivar that produces a concentration of toxin that reduces the fitness of a pest but does not, by itself, provide economically acceptable pest control.

B. *Bt* potato:

1. Does the Subpanel agree with EPA's assessment that CryIIIA is produced at high enough levels in *Bt* potato to be considered a high dose?

The Subpanel agreed that Monsanto's *Bt* potato produces a high dose relative to CPB. This conclusion was based on the fact that neonate larvae from a resistant CPB colony (over 200-fold resistance to *Bt*) could not survive on Monsanto's *Bt* potato.

2. What are the resistance management concerns if a "high dose" is not achieved in *Bt* potato?

The Subpanel indicated that for this pest, if a high dose was not achieved, there would be a very high risk of rapid resistance evolution.

C. *Bt* corn:

1. Does the Subpanel agree with EPA's analysis that CryIA(b) or CryIA(c) is produced at high enough levels in whorl stage *Bt* corn to be considered a high dose to control first generation ECB, but also that different expression events do not always produce a high enough dose to kill a sufficient number of second generation ECB larvae?

The Subpanel agreed that all current cultivars produce a high dose in whorl stage corn relative to ECB, but that some cultivars did not produce a high dose during the reproductive stage.

2. What are the resistance management concerns if there is not a high dose for full season control of ECB?

Research results currently in press (Onstad and Gould, 1998) examine the impact on resistance evolution of decreasing levels of mortality over the season. The conclusion from these papers is that the impact is contingent on the synchrony between ECB phenology in a given region and corn maturation. Examination of a number of years of phenology data from most corn growing states indicates that decreasing mortality over the season could cause resistance to evolve up to about 10 times faster than when a high dose is achieved all season. The model assumes a 5% refuge and a two generation ecotype.

3. Does the Subpanel agree or disagree that *Bt* corn without a high dose for second generation ECB can act as a partial refuge and reduce the likelihood that ECB resistance to *Bt* will develop?

The Subpanel believed that corn cultivars without a high dose do not provide a refuge for ECB.

D. *Bt* cotton:

1. Does the Subpanel agree with EPA's analysis that CryIA(c) is produced at high enough levels in *Bt* cotton to be considered a high dose to control TBW, but is not considered a high dose for either CBW/CEW or PBW?

The Subpanel felt that Monsanto's *Bt* cotton produces a high dose for TBW and PBW but not for CBW/CEW.

The conclusion that there was a high dose for TBW was based on mortality data for heterozygotes on Monsanto's *Bt* cotton. The conclusion that there was a high dose for PBW was based on field surveys that have never found any large larvae in Monsanto's *Bt* cotton. The conclusion that there was not a high dose for CBW/CEW was based on field data indicating that 5%-40% of susceptible CBW/CEW larvae can survive on *Bt* cotton.

Concern was expressed that if the problems seen in Australia in 1997 and 1998 with maintaining *Bt* toxin levels in cotton were also seen in the U.S., it is possible there may not be a high dose for TBW or PBW. It is, therefore, important to monitor expression levels in the field over a number of years and in varied environments and cultivars.

2. What are the resistance management concerns if there is not a high dose for CBW/CEW? PBW?

If high dose is not achieved, there are real concerns about resistance development in both species, especially with PBW in the western region where there might be problems with long season cotton and possible titer decline at the end of the season. If a high dose is not achieved for PBW, there could be rapid pest adaptation because there are almost no alternative hosts that could serve as a refuge, and western farmers have high adoption rates of *Bt* cotton which result in small non-*Bt* cotton refuges.

3. What special considerations should be made for CBW/CEW because of its inherent tolerance to Cry proteins and lack of resistance to most chemical insecticides compared to TBW?

Again, if high dose is not achieved, there are real concerns about resistance development. With CBW/CEW, where it is known there is not a high dose, refuges must be very large. Comments were made that more research is needed to determine just how large non-cotton refuges are.

E. All crop systems:

1. Given that there is significant variability in the expression of the toxin within a plant (i.e., varying expression in different plant parts), within a field (from plant to plant), or within a season (varying expression over time), how will a long-term resistance management strategy for each of the crop/pest situations be adjusted given the current dosage levels?

The Subpanel concluded that there was a real need to make sure that there is a robust refuge system that takes into account the entire cropping system, not just one crop and one pest.

Question 3: Secondary or Minor Pest Dosage Effects

For a complete definition of secondary pests for corn, cotton and potato, see Part I of this Report. In the context of integrated pest management (IPM), secondary pests are often defined as those that are either sporadic or build up in response to insecticides applied for a primary pest. However, in the context of *Bt* transgenic crops and resistance management, it is necessary to further refine what is meant by secondary pests. Specifically, the Subpanel defined a primary pest as the "targeted" pest for which a *Bt* crop is planted. Thus, a non-targeted pest (secondary) is one which is affected in one of two ways when a *Bt* crop is planted to manage a targeted (primary) pest: (1) one that is directly or potentially affected by a low to high dose expressed in a *Bt* crop but is of less economic significance than the targeted pest, or (2) one that is indirectly affected by a *Bt* crop through changes in insecticide use patterns. It is important to note that a non-targeted pest on one crop may actually be a targeted pest on another crop.

Examples of each of these types of non-targeted pests for the three crops are given in the definition in Part I of this Report.

The following questions are answered with the pest/crop framework described in the non-targeted pest definition of Part I of this Report in mind:

A. *Bt* corn:

1. In its evaluation of the initial *Bt* corn resistance management strategies, EPA identified a potential risk regarding the impact of CryIA(b)- and CryIA(c)-expressing *Bt* corn on CEW/CBW in those areas where CryIA(c)-expressing *Bt* cotton is grown. The Agency felt the impact would be greatest for *Bt* corn hybrids expressing CryIA(b) or CryIA(c) delta endotoxin in silk and kernels (at present this includes events MON810-, BT11-, DBT418-derived hybrids, but not Event 176-derived hybrids). Temporary sales restrictions on these *Bt* hybrids were imposed in the South because of the primary concern for resistant CEW/CBW to move from *Bt* corn to cotton/*Bt* cotton posing potentially significant problems in cotton/*Bt* cotton or in other crops affected by CEW/CBW. In addition to sales, distribution, and other restrictions, the EPA imposed research data and modeling requirements to evaluate the potential impact of *Bt* corn on *Bt* resistance management programs in areas growing corn and cotton. Based on the scientific information we now have, what if any refinements should be made to current resistance management practices in areas where *Bt* corn and *Bt* cotton could overlap?

The Subpanel had concerns about the phenology and movement of CEW/CBW between *Bt* corn and *Bt* cotton. For example, in the southeast U.S., the first generation of CEW/CBW larvae develop on wild hosts and whorl stage corn. The Subpanel believed that CEW/CBW is exposed to a high dose at this corn growth stage, but there are very little experimental data to support this assumption. In the second generation of CEW/CBW, most of the larvae are thought to feed on the ear stage of field corn. At this stage, the 176 corn event does not inhibit growth or cause significant mortality (according to most studies using isolines as controls). The other *Bt* corn events provide what might be considered a moderate dose, killing 65-95% of the larvae. The third generation of CEW/CBW then moves to cotton, soybean, other crops, and native vegetation. Furthermore, CEW/CBW will also be exposed to two closely related toxins, CryIA(b) (corn), and CryIA(c) (corn and cotton), which could also encourage development of cross-resistance, and potential loss of efficacy in both crops.

The current restriction on *Bt* corn in most areas of the southern U.S., is reasonable. One exception could be the high plains area of Texas. Here, *Bt* cotton will probably only be planted on limited acres, as it is not likely to be economical given the pest complex and yield potential. Thus, corn growers in this area should be able to plant *Bt* corn. Corn growers across the southern states should have similar rules relative to their total corn/cotton mix. One option might be to consider a joint restriction of both *Bt* corn and *Bt* cotton in some areas, rather than just restricting *Bt* corn.

This issue also raises the need to develop REGION-specific guidelines for resistance management plans for all *Bt* crops. Ideally, this should include a list of recommended, implementable structured refuge and resistance management options for growers, as well as a list of refuge options that should NOT be used. (See other Parts of this Report regarding region-specific, practical refuge guidelines).

2. What is the impact of *Bt* corn on other secondary pests such as fall armyworm, beet armyworm and SWCB?

Data regarding the impact of *Bt* corn on these and other non-targeted pests are limited and highly dependent upon the specific *Bt* corn event, *Bt* protein and/or promoter (see Ostlie *et al.*, 1997; Pilcher *et al.*, 1997). In areas where the geographic range of SWCB overlaps that of ECB, SWCB generally receives the higher management concern and thus is a primary or targeted pest of field corn. Concern in SWCB infested areas is elevated relative to ECB because individual SWCB larvae may tunnel 30 inches or more, and they also frequently girdle corn plants just above the soil level late in the season. Mechanical harvest may be impossible after girdled plants snap off at the site of girdling. Event 176 (*Bt* expression in green leaf tissue and pollen only) clearly does not provide a high dose against SWCB. For instance, hybrids containing Event 176 provided only 44% control in one Kansas field trial against heavy natural populations of this insect (Buschman *et al.*, 1997). BT11-based hybrids have consistently exhibited a high dose against SWCB and have provided virtually 100% control of this insect in small plot and large field university trials conducted in Kansas during 1996 and 1997. MON810-based commercial hybrids were first tested in small plots against SWCB by Kansas State University during 1997 (previous MON810 trials against ECB and SWCB in Kansas involved non-commercial lines; Buschman *et al.*, 1997). Small plot data collected during 1997 indicate that performance of MON810-containing commercial hybrids could not be differentiated from the SWCB protection provided by BT11 hybrids (Buschman *et al.*, 1998). The Subpanel was not aware of results, to date, on southern corn stalk borer (*Diatraea crambidoides* (Guenée)). Other Lepidoptera that should be minimally affected by current *Bt* hybrids include: black cutworm, western bean cutworm, stalk borers and armyworms (Ostlie *et al.*, 1997; Pilcher *et al.*, 1997).

3. Does the scientific data currently available indicate that refinements are needed for long-term resistance management strategies for ECB, considering CEW/CBW and other secondary pests?

Not at this time, at least for the midwest corn belt, because much of the basic moth movement and field studies needed to estimate optimum refuge size for CEW/CBW are lacking. This research is needed to verify when a high dose assumption may be met for SWCB so that appropriate refuges can be better defined for areas where both SWCB and ECB co-exist. Although there may be less selection pressure for some non-targeted pests, there is also a trade-off with CEW/CBW because it can attack *Bt* cotton as well. Thus, for CEW/CBW, e.g., in the mid-south and southeast U.S., it is likely that relatively large refuges of non-*Bt* crops will continue to be needed until more information is known about CEW/CBW movement and/or effective usefulness of alternate (non-crop) hosts.

4. Is there critical research needed for CEW/CBW, fall armyworm, SWCB, beet armyworm, southern corn stalk borer, to develop a comprehensive, long-term resistance management strategy for *Bt* corn?

Critical research is needed for SWCB and CEW/CBW, including: short-term movement, long-distance migration, mating behavior relative to movement (i.e., does mating occur before or after migration), estimates of initial allele frequencies for resistance genes, laboratory-selection studies to isolate resistance genes, mechanisms for resistance, fitness costs, refuge strategies, and monitoring strategies that work for each. Some resistance monitoring is in place for CEW/CBW (Hardee, USDA-ARS); monitoring protocols need to be developed for SWCB. Although it could be argued that more research is needed for other non-targeted pests, these two pests have the highest priority.

B. *Bt* cotton:

1. Does the Subpanel agree with EPA's analysis that there is a risk regarding the impact of CryIA(c) expressing *Bt* cotton on secondary pests such as cabbage looper, soybean looper, saltmarsh caterpillar, cotton leaf perforator and ECB?

The Subpanel agreed that some of these pests may be at risk.

2. What role do regional differences play with these concerns and how would a long-term resistance management strategy be modified to address these concerns?

Regional differences are very important, not only on a broad scale but even within traditional regional boundaries. For example, differences between the southeastern and southwestern U.S., are clear, with PBW as the dominant, targeted pest in the southwest deserts. However, within a region such as the southeastern U.S., there are many differences among pest groups. For example, cotton aphid is more important in Mississippi than in Georgia. ECB is of greater concern in North Carolina than in the mid-south. Differences occur because of climate, production practices (irrigation vs. dryland), presence of alternative hosts, etc.

3. How would the current resistance management strategy for TBW, CBW/CEW, and PBW, need to be adjusted to consider these other secondary pests?

For non-targeted pests that may be exposed to low or moderate doses (see the Secondary Pest Definition in this Report), a larger refuge may be required.

C. *Bt* potato:

1. What secondary pests, if any, are of concern for long-term resistance management in *Bt* potato?

There is no scientific basis at this time to believe that other pests in the potato ecosystem will develop resistance to *Bt*, or be adversely affected by *Bt* potato.

Question 4: Mechanisms and Genetics of Resistance

A. Does the Subpanel agree that protease inhibition, behavioral modifications, and binding site modifications are all important mechanisms of *Bt* resistance for the targeted pests of *Bt* potato, *Bt* corn, and *Bt* cotton? Are there other important resistance mechanisms to be considered?

Yes. Although Heckel (1994) identified eleven potential mechanisms for the development of resistance, these three are the most important and probably represent the primary resistance mechanisms.

1. Do any of these mechanisms, other than binding site modifications, increase the likelihood for cross resistance?

Yes, it seems likely that resistance based on proteases could provide more wide-spectrum resistance.

2. In field populations, which of these mechanisms is likely to contribute to the development of resistance?

Strong, specific resistance resulting from binding site modifications has been identified under both laboratory and field selections and is considered to be the primary mechanism for *Bt* resistance. These mutations are mostly recessive. However, behavioral modifications such as changes in phenology may allow insects to avoid highest *Bt* levels and reduce effective dose exposure. Likewise, protease modifications can also reduce effective dose. These mechanisms may lead to reduced control of insects that are heterozygous for partially recessive resistance alleles and hasten the development of strong resistance. The Subpanel noted that resistance mechanisms based on protease activity are likely to be dominant.

B. What experiments can be devised to examine mechanisms of resistance (e.g., binding site modifications, behavioral modifications, protease inhibitions) that would be of value in developing long-term resistance management strategies?

Research is needed to analyze the behavior, physiology and biochemistry of resistance mechanisms. Feeding cessation and gut recovery mechanisms are important. The Subpanel noted that analysis of binding assays are much more difficult in CPB than in lepidopterans. More research into the biochemistry of *Bt* resistance mechanisms should be continued, but the Subpanel noted that in most cases, the data will not be available in time for regulatory decisions. Identification of molecular genetic markers for *Bt* resistance genes could provide important tools for genetic analysis of resistance alleles under both laboratory and field conditions.

C. Does the Subpanel agree with the EPA's conclusion that *Bt* resistance in laboratory-selected colonies may not reflect the rates and mechanisms of resistance generated in field populations, but *Bt* resistance in laboratory-selected colonies is useful for the study of potential risk of resistance, physiological and behavioral mechanisms of resistance, cross resistance, genetics, stability, fitness costs, and the development of methods for monitoring, managing, and delaying resistance?

Yes. Laboratory-selected *Bt* resistant strains are critically important for estimation of allele frequency, physiological and biochemical analysis of resistance mechanisms, cross resistance and genetics. The Subpanel noted that caution should be exercised in using laboratory-selected colonies for estimating fitness costs and the development of resistance management practices because of the multiplicity of other factors.

D. What accounts for the variable susceptibility to *Bt* toxins in field populations for the key pests of potato, corn, and cotton: (1) CPB, (2) ECB, (3) TBW, (4) CEW/CBW, and (5) PBW?

The Subpanel noted variations in: (1) behavior, (2) feeding interruptions in susceptible insects, (3) distribution of receptors or proteases in the gut that may be affected by regional differences in diet, etc., (4) active sites, (5) gut recovery, and (6) development of septicemia and death. Some reported variations in *Bt* susceptibility may be due to differences in assay methods.

E. Discuss the impact of fitness costs (e.g., growth suppression, reduced fecundity):

1. Associated with sublethal doses of *Bt* toxins expressed in *Bt* crop (e.g., *Bt* potato, *Bt* corn, and *Bt* cotton).

2. Associated with adaptation (resistance) to *Bt* pesticides.

The Subpanel noted that delayed development can result in genetic isolation of *Bt* affected insects potentially reducing the effectiveness of refuges. It can also affect key issues in resistance management, including mating ecology, overwintering success, and movement of adults and larvae. The relationship between resistance genes and fitness costs may not be straightforward. Sometimes genes with low fitness costs may be important in the field.

F. If a company can produce evidence that *Bt* toxin (X) has an alternate binding site or mode of action to another *Bt* toxin (Y) for a given pest, is there any scientific reason that *Bt* microbial products with toxin X should not be used in the resistance management for a *Bt* crop expressing toxin Y?

Probably not. However, there could be mechanisms such as protease inhibition that could result in some cross resistance between toxins with different modes of action.

G. Discuss the relevance of diamondback moth resistance to *Bt* microbial pesticides in relation to the potential for *Bt* resistance to develop in the targeted pests of *Bt* potato, *Bt* corn, *Bt* cotton, and other *Bt* crops?

Although the diamondback moth (*Plutella xylostella* (Linnaeus))(DBM) may provide a reasonable model for analysis of *Bt* resistance in insects at the molecular and/or physiological level, the Subpanel emphasized that any comparisons, based on the results of DBM studies to targeted pests of transgenic *Bt* crops, should be done with caution. At least two reasons for limited extrapolation across species are: (1) differences in response of geographically isolated DBM populations world-wide to *Bt* selection pressure in the field (Hawaii, Phillippines, Florida) (Tabashnik *et al.*, 1997), and (2) different gut pH toxicity mechanisms between Lepidoptera and Coleoptera (Koller *et al.*, 1992), either of which may confer different inheritance mechanisms.

Question 5: Cross-Resistance Potential

A. Do you agree with EPA's analysis that the potential to develop cross-resistance to a number of Cry proteins exists for a number of insect species, including CPB, ECB, TBW, CEW/CBW, and PBW?

Yes.

B. Does the Subpanel agree or disagree that the cross-resistance potential can be estimated using the results of binding studies for a particular pest and amino acid sequence homology?

Yes and no. In some instances (*Moar et al.*, 1995, Gould *et al.*, 1992, Macintosh *et al.*, 1991) this relationship does hold up, but not in all. Therefore, caution is advised in applying the concept across all combinations of pests and amino acid sequences.

C. Which *Bt* derived toxins (through shared binding sites or other means) and targeted pests are most at risk to develop cross-resistance?

When resistance has developed in the field or laboratory to either CryIA(c) or CryIA(b) in Lepidoptera, cross-resistance to the other toxins usually results. However, the level of cross-resistance varies broadly in various pests. In general, the greater the similarity between toxins, the greater the chance that cross-resistance will be seen. However, this relationship does not always hold.

The Subpanel considered CEW/CBW to be the most likely Lepidoptera to develop cross-resistance in areas where both *Bt* cotton and *Bt* corn transgenic crops are deployed within the geographic range of this pest.

D. What scientific factors should EPA consider when other crops (e.g., vegetable crops) containing the same Cry proteins have the potential for cross-resistance?

EPA should consider the same factors that they currently consider in their current resistance management programs for *Bt* crops. Meeting requirements for the high dose/refuge strategy is especially important. In other words, the different crops containing *Bt* toxins should be considered as an integrated whole when making decisions regarding temporal and spatial refuge, IPM factors, monitoring, communication and education, etc.

E. Does the Subpanel agree with EPA's analysis that pyramiding two *Bt* genes with different modes of action is a powerful tool in managing resistance? Discuss how pyramiding strategies using two different Cry proteins affect the potential development of cross-resistance and how a long-term resistance management strategy would be affected.

The Subpanel agreed, as long as the two genes that are introduced into the plant are expressed in the same temporal and spatial pattern. Differential expression of two transgenes could lead to more rapid resistance development to both toxins, because all insects would not necessarily be exposed to both toxins simultaneously. For the same reason, pyramiding also provides lower risk of resistance development than sequential deployment of toxins.

1. How does the use of two toxins with different receptor sites, such as Cry9(c) and CryI(A), in *Bt* corn or other *Bt* crops, affect the potential development of cross-resistance?

The answer depends on the resistance mechanism. If resistance is mediated by target site alterations, then using toxins that affect two different sites will prolong resistance development. If other types of resistance occur (metabolic, gut recovery, etc.), the potential for cross-resistance may not be affected by target site specificity.

2. How does the use of two toxins which may share the same receptor site, but may also have additional unique receptor sites, such as CryIF and CryI(A) or Cry9(c) and CryIC, in *Bt* corn or other *Bt* crops, affect the potential development of cross-resistance?

Although it seems that the ability of a toxin to interact with more than one target site would reduce the potential for cross-resistance, this situation is difficult to predict with any certainty. One cannot rule out *a priori* other, non-targeted site mediated types of resistance that could yield cross-resistance independent of the additional sites. However, one could point to the anecdotal evidence from synthetic insecticides with multiple modes of action. Resistance to these compounds tends to develop slowly compared to compounds with a single mode of action. Note also that while binding is essential for intoxication, binding kinetics do not constitute all cross-resistance mechanisms.

F. As noted in EPA's analysis, the literature indicates that CryIIA delta endotoxin has a voltage-dependent mechanism for pore formation and that CryI and CryIII delta endotoxins have an ion-dependent mechanism for pore formation. However, some scientific evidence indicates low levels of broad cross resistance between CryIIA and CryIA delta endotoxins (e.g., beet armyworm and TBW). How does the difference in biochemical mechanism affect the potential development of cross-resistance (laboratory vs. field)?

If the resistance mechanisms are based on the mechanism of pore formation, then it is predicted that toxins with different pore forming processes could perhaps prevent cross-resistance. Therefore, even though two toxins may share the same binding site(s), cross-resistance may not result between them. However, if the resistance mechanism were independent of pore formation, no advantage would result.

G. The scientific literature indicates that even though two proteins, *Bt* toxin X and *Bt* toxin Y, may share the same binding sites, insects resistant to *Bt* toxin X remain susceptible to *Bt* toxin Y. For example, two separate studies of resistance in TBW found no relationship between resistance to CryIA(b) or CryIA(c) and toxin-receptor binding. Discuss the relevance of this information to predicting the potential to develop cross-resistance.

The Subpanel noted that work on DBM has shown that cross-resistance did not occur between two toxins that shared a major binding protein (Ballester *et al.*, 1994). This work demonstrated that binding site may be only part of the cross-resistance picture. A better understanding of the *Bt* intoxication mechanism may help to explain this phenomenon (see Heckel, 1994). It includes *per os* ingestion, ingestion/intoxication initiation that somehow results in feeding cessation, various levels of protein processing within the gut, and transport to the active site. Factors such as the gut environment including pH and protein composition, binding kinetics, pore formation kinetics, cell lysis dynamics, septicemia and death all affect this process. This complex cascade of events and feedback mechanisms could contribute either positively or negatively to cross-resistance without involving binding. Therefore, caution is advised in invoking receptor binding as the only cross-resistance mechanism.

Question 6: Predictive Modeling

A. Based on what we know about predictive modeling in developing resistance management strategies for *Bt* potato, *Bt* corn, and *Bt* cotton, how can these models best be used to develop long-term resistance management strategies?

1. What are the critical assumptions made in these models (e.g., random mating, single or multiple pests, initial resistance allele frequency)?

Models have limited ability to predict how many years it will take for resistance to develop in a selected insect population, not in the least because knowledge of certain key parameters (e.g., genetics of resistance) are inherently limited prior to the evolution of resistance. Models can be used more profitably to evaluate the relative effectiveness of different strategies (e.g., one strategy is likely to delay resistance twice as long as an alternative strategy). Models also serve an important function as a research tool in that they force one to formalize available knowledge, data and concepts and identify (through sensitivity analysis) those parameters where information is most needed (prioritization of research needs). Perhaps the best example of this process is the empirical research done on larval movement following the publication of Mallet and Porter (1992).

Validation of models can at least be accomplished by comparison of the population dynamics of the models to actual field data. Recent work in resistance modeling has shown that inclusion of spatially explicit population dynamics (spatial ecology) in addition to population genetics is critical, suggesting that such validation is important. At present, validation of the population genetics components of such models is limited to comparison with historical events.

2. How are these models used to estimate the effective refuge size and structure needed to manage long-term resistance? Based on current research data, how can the existing models be improved?

The Subpanel was split on this issue. There was no doubt expressed by the Subpanel that the models demonstrated that refuges were critical for resistance management, but some Subpanel members felt that they provided little information on the actual refuge size necessary. The Subpanel suggested that the models made best use of available data and conservative interpretation of modeling results should be used as a guide for determining refuge size until more complete information is available. The Subpanel assumed that its charge from the EPA was to comment on the best resistance management strategies considering the effects of economic and logistical feasibility on grower acceptance.

3. What supportive field information has been collected to validate these models?

Numerous studies have been conducted by various registrants. However, these reports are proprietary and many have not yet been summarized or made available to the public. The Subpanel recognized that field resistance development at this early stage to *Bt* plant deployment was unlikely regardless of the resistance management program deployed.

4. What additional field information is necessary to validate the predictive models?

Information on population dynamics, initial gene frequencies, and insect movement must continue to be collected. Monitoring studies may collect data that will permit retrospective modeling, leading to improvement of models for future work.

B. How do predictive models consider the fitness costs to "resistant" survivors of exposure to *Bt* crops, including reduced fecundity, viability, development time, and growth weight?

Fitness costs are generally considered from a conservative approach; that is, the potential advantages to resistance management from fitness costs are limited as fitness costs have not always been associated with resistance alleles. In some cases, however, fitness costs in terms of developmental delays may increase the rate of resistance evolution, and such fitness costs must be considered in detail. A definition of "fitness costs" is provided in Part I of this Report.

C. Based on what we know about the initial resistance allele frequency for TBW, how can this information best be used to improve the predictive value of existing models and refine current resistance management strategies?

The estimate of initial gene frequency for TBW has been incorporated into many current models, and it has, in general, not dramatically altered the relative ranking for the different strategies examined.

D. How can the initial resistance allele frequencies be measured for other targeted pests of *Bt* potato, *Bt* corn, and *Bt* cotton and be used to improve the predictive value of existing models and refine current resistance management strategies?

Initial resistance allele frequencies can be measured by a number of methods. Gould *et al.*, (1997) selected a resistant colony under laboratory conditions and was able to estimate the frequency of resistance alleles in field populations. A method proposed by Andow and Alstad (1998), utilizing inbred lines, would recover multiple resistance alleles. Both of the techniques are limited to recovering alleles that are present at a frequency of 0.001 or above. A new method using *Bt* sentinel sweet corn, employed by Hutchison (Andow and Hutchinson, 1998; Hutchison, unpublished data) has the potential of estimating the presence of resistance alleles at much lower frequencies.

Question 7: Deployment of Alternate Modes of Action

A. As noted in previous EPA analyses, the March 1995 Subpanel meeting (FIFRA, 1995), and EPA's issue paper for this meeting (EPA, 1998), deployment of toxins with alternate modes of action is important in developing a long-term resistance management strategy for any *Bt* crop. Are there any alternate modes of action which would not be an effective resistance management tactic?

No, as long as control tactics employing alternate modes of action are not used to suppress pest populations in the refuge. There was discussion regarding techniques such as sterile male releases or mating disruption that may only have a neutral effect on resistance development, if the effect was applied evenly across both *Bt* plants and refuge. However, if the strategies can be effectively implemented only in areas planted with *Bt* transgenic plants, there would be a net benefit to resistance management. It was also mentioned that the use of mating pheromones in areas utilizing *Bt* plants might actually serve to recruit susceptible homozygotes originating from a refuge planting.

Although there may be benefits to a resistance management program, there was also concern expressed regarding acceptance by growers because of the added expense of employing a second control strategy when they have already invested in the *Bt* technology.

B. Discuss how a resistance management strategy would need to be adjusted when new *Bt* genes or other novel genes are introduced into corn, potato, cotton and other crops.

The Subpanel focused primarily on the deployment of pyramided genes into the various crop plants and the effect on refuge size. Although there was general agreement that pyramided plants would have a positive effect on resistance management, there is uncertainty associated with establishing the refuge, and a conservative approach was encouraged. There was discussion regarding the benefits of *Bt* plants that express a high dose for two different toxic genes, and it was agreed that companies should be provided incentive for producing products that are more effective in managing resistance (e.g., new corn/cotton products expressing toxins that would alleviate concerns for CEW/CBW resistance development in the southeastern U.S.).

C. What are the advantages and disadvantages of the following methods of introduction of *Bt* toxins into the environment? 1. mosaics? 2. alternations? 3. mixtures? 4. pyramids?

It was pointed out that in cases of synthetic chemical insecticides when mixtures of compounds were utilized, if one chemical degrades faster that the other, there is no net benefit to resistance management. It was agreed that two genes that are expressed at high doses and are utilized in a pyramid scheme offer distinct advantages over mixtures and alterations, but these genes must have season-long expression to be effective. If such plants become available, the advantage would only be obtained if plants expressing a single toxin were no longer utilized. If mixtures of pyramided plants were utilized in conjunction with plants expressing a single gene, the effect would be to offer the exposed population a "stepping stone" for resistance to develop ultimately to both toxins. Therefore, it was important that regulatory decisions regarding registration of pyramided genes consider the other registrations of single gene technologies.

From a sociological perspective, it was identified that growers might not see the benefit of using plants that have pyramided genes and might be less likely to adopt the technology if a single gene is performing satisfactorily.

There has been some discussion about the utility of planting a portion of a crop's acreage in cultivars with one *Bt* toxin (e.g., CryIA(c)) and another portion in cultivars with a second distinct *Bt* toxin (e.g., Cry9(c)). Results of computer simulation models (e.g., Roush, 1989) indicate that such plantings are unlikely to offer significant delay in the evolution of resistance to the toxins. If there was no refuge for the insects exposed to the toxins, resistance is expected to evolve rapidly. When two distinct toxins are available, model results indicate that it is much better to pyramid the two toxins and have a refuge available.

Question 8: Integration of *Bt* crops with IPM

A. Does the Subpanel agree with EPA's analysis that the deployment of *Bt* potato, *Bt* corn, and *Bt* cotton should be integrated into IPM programs?

Yes.

Comments by Subpanel members indicated that growers need additional decision-making tools which help them determine when the risks of economic pest damage become great enough to justify the use of supplemental pest management strategies. Such information is necessary for non-targeted pests which are not completely suppressed by the *Bt* technology. Significant educational efforts will be necessary to ensure that technology transfer includes practical information to guide long-term and sustained implementation of the technology at the user level.

Specific crop hybrids are selected by growers for a combination of reasons which have local relevance, and rarely is selection based solely on insect resistance. Important criteria guiding purchase decisions frequently revolve around proven yield potential (probably the most important factor), adaptation, intended use, cost, harvest characteristics, pest tolerance, and many other factors. Decisions involving identity and quantities of seed (e.g., relative amounts of *Bt* and non-*Bt* corn) may be made as early as November of the previous year, particularly if the seed is expected to have limited availability. Management advice and regulations need to provide realistic lead time if future practices are to be adopted at the grower level.

B. Does the Subpanel agree with EPA's analysis that scouting, monitoring, and insecticidal treatments will have to be altered when using *Bt* crops? How have IPM practices (e.g., crop rotations, destruction of overwintering material, pheromone traps, flame-torching, reduced tillage, planting dates, plant varieties) been modified in conjunction with

development of a long-term resistance management strategy for *Bt* potato, *Bt* corn, and *Bt* cotton?

Responses to this question seemed to vary by crop. Little change in scouting was predicted for most corn pests with the exception that some high expressing cultivars may require decreased corn borer scouting effort. Some concern was expressed that potato producers may be narrowing their consideration of pest management options in some areas where CPB is the key pest needing control. In *Bt* cotton, scouting for non-targeted pests should be intensified in cases where insecticides have been used in the past in non-*Bt* cotton (e.g., for tarnished plant bug in the midsouthern U.S.).

There seems to be increased awareness among stakeholders (farmers, consultants, and seed companies) that resistance management strategies are justified and are essential components of production systems that utilize transgenic hybrids. There is broader recognition and acceptance among these stakeholders that good product stewardship involves some form of refuge with non-*Bt* hybrids. For some crops, scouting and management options will be modified, and growers need to be prepared to look beyond individual commodity management as we attempt to harmonize risks and benefits.

C. How do refuges fit in with IPM programs?

Generally, refuges are viewed as fully compatible with IPM programs. Some producers may mistakenly believe that use of a *Bt* crop will solve their insect management problems, thus causing them to drop their efforts at maintaining a broadly balanced pest management program. Compliance issues were discussed in terms of the extra effort associated with verifying that truly effective refuges are established (those that provide for adult emergence patterns which facilitate mating among resistant and susceptible pest genotypes). Refuges that correspond to transgenic planting need to be managed similarly (planting date, fertility, irrigation, maturity, etc.). Issues of proximity (in-field, near field, etc.), timing of establishment, and percentage of area required, will likely generate the most questions from growers considering implementation of refuges.

The search for practical, grower-accepted refuge planting patterns has probably focused more attention on IPM in certain situations. The concept of a refuge is sufficiently foreign to users that they need more detailed explanations and justifications which lead to enhanced learning about pest management concepts in general.

Gene stacking involving herbicide resistance paired with insect resistance is likely to increase the complexity in situations where refuges are required or desirable. Availability of matched non-expressing and transgenic-trait-expressing hybrids with similar agronomic characteristics will be important aspects affecting the practical implementation of refuge recommendations.

Bt corn has brought about an increased awareness of damage caused by ECB. Having prevented ECB yield losses for the first time, many corn growers now appreciate the degree of damage this insect caused in previous years. Producers seem to be more willing to consider employing refuge plantings as long as insecticidal spray options are available if pests in the refuge planting reach economically threatening levels. Insecticidal treatment of refuges is an option that is particularly needed in regions where SWCB and occasional outbreaks of other pests (fall armyworm, etc.) occur. There was some concern that certain types of refuges that allow direct comparisons between *Bt* and non-*Bt* hybrids may initially be accepted by growers and then dropped when the novelty of this direct comparison wears off. The Subpanel recognized the importance of intensive educational efforts to convince growers to adopt practical steps designed to create and maintain a suitable local refuge.

Bt insecticides in any form (whether plant-incorporated or as sprayable microbials) should not be used to control pests in refuges.

D. How will existing quarantine programs (e.g., pheromone trapping, destruction of overwintering material, sterile male release) for PBW affect the efficiency of the *Bt* cotton refuge in producing susceptible individuals? Are existing quarantine programs adequate to manage PBW resistance in *Bt* cotton without the use of a refuge?

PBW is a well-established pest in Arizona and is not the subject of a quarantine program. Pheromone trapping for PBW is conducted throughout Arizona by the Arizona Cotton Research and Protection Council, but this monitoring will not impact production of susceptible moths in refuges. Additional measures used to control PBW in Arizona, such as enforcement of plowdown dates, trap crops or early crop termination, should not negatively impact production of susceptible individuals from refuges. Pheromone products used to disrupt PBW mating, if used early in the season, could reduce numbers of susceptible moths produced in refuges. However, existing regulations prohibit the use of any chemicals in refuges that suppress PBW. Discussion indicated that high dose strategy should still be paired with a valid refuge management strategy, even in the PBW areas of the southwest U.S.

PBW is under a population regulation program in California's San Joaquin Valley, where a long-standing collaboration between the California Department of Agriculture and the USDA annually releases sterile PBW moths to eradicate the small numbers of adults that inevitably infest southern San Joaquin cotton fields. At present, this has negligible impact on managing PBW resistance to *Bt* cotton because of the very limited use of *Bt* cotton in the San Joaquin Valley.

Question 9: Refuge Strategies

The Subpanel considered refuge strategies an especially important topic. A list of research issues relevant to this topic can be found in Part III of this Report.

A. General considerations:

1. Does the Subpanel agree with the EPA's analysis that a structured refuge is considered essential in the development of a long-term resistance management strategy for *Bt* potato, Bt corn, and *Bt* cotton?

Yes, for all three crops.

2. Are there other types of refuges, besides structural, that should be considered (e.g., temporal, alternate host crops or weeds)?

Yes. For example, rotating *Bt* plantings with non-*Bt* plantings was noted as an especially important temporal refuge tactic. Note related comments in Responses to Questions 9A3 and 9F6 of this Report.

3. How will varying environmental conditions and geographic regions affect refuge designs?

The Subpanel considered regionalization an important refuge issue for all three crops. Discussion related to these issues occurred within this question and a number of other questions.

General comments: Stable refuges over time are more effective than non-stable refuges. Related to this, refuges that stay in one place are more effective than refuges that require insect movement. Refuge stability was discussed extensively and was considered a critical element. A spatial model (Peck, 1997) was cited as an important tool to evaluate effectiveness of various refuge/*Bt* crop configurations.

Corn: Irrigated and non-irrigated cropping systems were cited as specific examples where regional considerations were important. Also, in areas with SWCB, a spray option should be available to producers.

ECB production in alternate hosts, weeds and small grains occurs in central Iowa. At times these populations can be relatively large, but they are influenced by crop phenology and environmental conditions. They should not be considered reliable sources of ECB refuge. The concept of a "super" refuge was discussed. There is the possibility of producing large numbers of ECB and perhaps other pest insects in small confined areas (Andow and Hutchison, 1998; Hellmich, unpublished data).

Discussion about formulating specific regional refuge recommendations for producers led to the conclusion that specific recommendations for each region was not a desirable approach. Rather a list of options that producers could choose to adapt to their unique farming practices was considered better and recommended by the Subpanel. These options could be complemented with specific recommendations about which farming practices producers should avoid, i.e., those that are detrimental to resistance management while considering the growers' needs and economics.

Cotton: Heliothine wild hosts are common, but the importance of these hosts as refuge has not been quantified nor characterized as stable. Spring alternate hosts in the southeast U.S., seem to be an exception and are considered to be relatively stable. Soybeans often are not an effective CBW/CEW host because CBW/CEW survival is influenced by plant senescence. In the southwest U.S., there are no significant wild hosts of PBW.

Potato: Each region has a different alternate host profile for CPB. For example, nightshades and horse nettle impact second generation CPB in the midwest U.S. Alternate hosts are not as important in the western U.S.

4. What scientific data are necessary to determine the size of an adequate refuge?

During its deliberations, the Subpanel developed a list of Research Needs (see Part III of this Report) and a definition of "high dose" (see Part I of this Report), and these are integral to this response.

Sensitivity analyses of model parameters suggest that initial frequency of resistance alleles, survival rate of heterozygotes, and insect population dynamics are some of the genetic/biological factors that are important. The Subpanel discussed in great detail methods to estimate initial resistance gene frequencies which included *Bt* sentinel sweet-corn plots (Andow and Hutchison, 1998), F2 screen (Andow and Alstad, 1998), and screening against test stocks (Gould *et al.*, 1997).

In discussion of high dose assumptions for many of the events, Subpanel members were concerned that some of the corn events did not satisfy high dose criteria. The problem with *Bt* cotton not satisfying high dose criteria for CBW/CEW also was referenced and led to a definition of high dose (see Part I of this Report).

Current models use information from population genetics and insect population dynamics, but research is needed to better define population parameters of all the major insect pests. Spatial models require better information about insect movement, mating behavior, oviposition patterns, and aggregation behavior.

Plant titre variation (within plant, between plant and seasonal) was considered another important factor for determining size of an adequate refuge. Important economic factors for determining refuge size included planning horizon and technology fees.

B. Discuss the importance of refuge placement (e.g., part of the transgenic field or at an external site from the transgenic field).

1. Can completely random mating be expected with an out-of-field (external) refuge?

Random mating is an important assumption, and impact on resistance management is always negative whenever this assumption is violated. Non-random oviposition could be either positive or negative. For example, preferred mating on refuge plants could increase the relative value of refuge; the reverse could decrease the relative value of refuge.

Each species should be evaluated on a case by case basis regarding the assumption of random mating. Delayed development caused by exposure to *Bt* toxins could lead to non-random mating which could be especially problematic for insects that have two or more generations per year.

2. **What research has been undertaken to address this issue that EPA has not identified in its analysis?**

No specific examples were noted.

C. Refuge management:

1. **Discuss the implications for resistance management of a managed refuge (i.e., treated with insecticides or some other control measure for key pests) versus an unmanaged refuge (i.e., untreated for key pests).**

Discussion on this question was linked with that of the following question. See Subpanel Responses to Question 9C2.

2. **Discuss the potential impact of refuge management on the availability of susceptible insects and their movement between the refuge and the *Bt* crop (to ensure random mating with possible resistant insects).**

The bottom line for refuge management is that if few or no susceptible insects are produced, then the refuge component of a resistance management strategy fails. The economics of refuge management will vary depending on crop and pest species. Cotton, potato, and corn refuges in areas with SWCB could be problematic. Perhaps growers in these cases will need incentives to implement resistance management that is not economical in the short run. In the case of corn, where in some regions ECB treatments are uncommon, untreated refuge should be an economical option. This is especially true when a long-term approach to economics and resistance management are considered (Hurley *et al.*, 1998). Corn entomologists are concerned, however, that grower management of ECB could change drastically when the "hidden losses" of ECB are recognized from comparisons between *Bt* hybrids and non-*Bt* hybrids. If this occurs, grower incentives might be necessary in corn.

In the case of potato, a conservative approach to refuge size and treatments of refuge was recommended. Much of the biology of the CPB that would influence refuge size and structure is not well understood. In particular, more research is needed on CPB recruitment, movement, overwintering, and field ecology (especially landscape influences). Regional differences for all of the above could occur. Other concerns about CPB resistance management included *Bt* delayed development and the effects this could have on random mating, and effects environmental influences have on CPB movement. In general, CPB populations appear to be stable, i.e., not very mobile, but this changes drastically under some environmental conditions. Finally, imidacloprid (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneaminein, Bayer Corp., tradenames include Admire and Provado) is recognized as a very effective CPB insecticide that virtually eliminates CPB from treated refuge. Widespread use of imidacloprid is incompatible with CPB refuges for *Bt* potato.

In cotton, many of the same concerns exist as in corn. With a 20% controlled refuge, few or no susceptible insects may be produced, and thus the refuge strategy may fail. Thus, it may be necessary to restrict efficacy of insecticides to no more than 90-95% control in the 20% sprayed refuge. Cotton entomologists are also concerned that grower management of CBW/CEW in both the *Bt* and refuge areas could increase, such as in automatic or recommended pyrethroid sprays when egg "thresholds" are reached after growers experience increased yields following such sprays. If this occurs, increased changes of pyrethroid resistance will occur in both *Bt* and non-*Bt* cotton.

The Subpanel discussed key requirements of refuge. Fundamentally, refuge should produce at least 500 susceptible insects for every resistant insect that survives in the *Bt* crop (see Attachment I of this Report), but higher ratios should be encouraged when possible. Deviations from random mating alone are always detrimental, but when coupled with non-random oviposition, can in fact delay resistance evolution. In short, if more eggs are laid in the refuge, the refuge population grows more rapidly, and even though individuals are less likely to mate at random, there are many more individuals so that resistant moths in *Bt* fields are less likely to mate with each other. If non-random mating is coupled with non-random oviposition, there is an optimal value of non-random mating that delays resistance longer, and in some models much longer, than random mating. The Subpanel recognized the importance of refuge size and structure for the above requirements. They noted that additional research for each cropping system and pest was needed before specific recommendations could be made. Until then, a conservative approach (i.e., one that maintains more susceptible insects) toward refuge management was recommended.

3. Discuss the potential impact of refuge management on beneficial insect populations.

The Subpanel recognized the importance of beneficial insects to the whole system of refuge management (Gould *et al.*, 1994). Beneficial insects that reduce populations of refuge insects could reduce the value of the refuge. On the other hand, if beneficial insects are killing resistant insects, this could be an advantage. The Subpanel recognized that beneficial insects add a layer of complexity to resistance management that could be very important. More research is needed to resolve these questions.

D. Seed mixes as refuge:

1. Does the Subpanel agree with EPA that seed mixes should be eliminated from consideration as a viable refuge option for CPB control in *Bt* potato, and ECB and CEW control in *Bt* corn, and CBW and TBW control in *Bt* cotton?

Yes.

2. Does the Subpanel agree with EPA's analysis that a seed mix option may be viable for PBW?

Seed mixture option for PBW is viable in Arizona. Producers and researchers, however, should be aware of a possible resurgence of heliothines. In New Mexico, seed mixtures are not a viable option because of the presence of TBW and CBW/CEW.

E. Refuge structure--potato:

1. Does the Subpanel have any scientific evidence that indicates that a 20% structured refuge is an inadequate refuge for CPB resistance management in the long-term?

In a 20% refuge that is sprayed, especially with imidacloprid, most if not all of the refuge beetles could be killed. IPM principles should be applied within the refuge to reduce selection pressure on insecticides and *Bt* potato. Structured refuge, especially in terms of distance, must be considered in an IPM strategy. Economic injury levels for CPB on potato (<20% foliage damage) are well understood. How to layer refuge on top of this is not.

2. What factors should EPA consider if tomato or minor crops, such as eggplant, are also producing the CryIIIA toxin?

All biological factors discussed for previous pests should be considered. Additionally, the interface of these crops with *Bt* potato and other plants within the ecosystem must be considered.

F. Refuge structure--corn:

1. EPA has requested testing of structured and temporal refuges assuming a slow adoption of *Bt* corn (less than 10%) in 5 years. Is there now scientific evidence that one option is better than the other for a long-term resistance management strategy?

Structured refuges are preferred**.**

2. **Discuss the "halo effect" for *Bt* corn planted in close proximity to non-*Bt* corn and its implications for refuges.**

The Subpanel provided a definition of the "halo effect" in Part I of this Report. The "halo effect" is not unique to *Bt* corn. Other control measures could produce this effect, especially in crops where control percentages are high. The halo effect could have utility for strips but it is less likely to have utility for blocks. The Subpanel believed that although interesting from a biological perspective, the halo effect will not likely be a driving force in producer decisions. Researchers should note that a halo effect could be negative if it depletes refuges.

3. **Is there any scientific evidence that indicates whether a 5% unsprayed or 20 to 30% sprayed refuge is inadequate for long-term resistance management?**

Although no field data are available, most models suggest that 5% unsprayed refuge is inadequate. The same can be applied to 20-30% sprayed refuge, when control is high. Cage studies on DBM in New York suggest that a refuge does delay the development of resistance and that producers should err on the conservative side when implementing a refuge. The Subpanel noted that a 1995 Subpanel of the FIFRA Scientific Advisory Committee (1995), recommended refuge as one of the most important elements of a sound resistance management program. It is not possible to know if refuges successfully delay resistance until the refuge strategy succeeds or fails in the field. Two years of field data are not sufficient to evaluate the concept.

4. **Is there any scientific evidence that indicates whether under continuous non-*Bt* corn refuge acreage sprayed intensively with chemical insecticides that the refuge size would need to be increased, for example to 40%, to compensate for larval mortality?**

No field data are available, and the central tradeoff is susceptible insect production which requires at least some plant damage and economical crop production. This susceptible insect and crop production equation will vary from crop to crop and for many factors within each cropping system. In general, whenever the refuge is sprayed, the amount of refuge should be increased to compensate for larval mortality. Percentage refuge increases required to compensate for insect mortality are difficult to determine.

In the corn system, refuge size should increase if producers spray for insects. Insecticides used on ECB in corn range from providing 70-90% control for first generation to 50-75% control for second generation. Even heavily sprayed refuge corn will produce ECB. The numbers will vary tremendously depending on spray timing, environmental conditions and ECB ecotype, and the refuge size should be flexible.

5. Are variations in refuge size and structure appropriate for different regions of the country, especially northern and southern corn growing regions?

Yes.

6. Can weeds and other alternate crops growing in the same area and season, outside of *Bt* corn fields, serve as refuges for ECB, CEW/CBW, other corn pests controlled or suppressed by Cry proteins? What scientific evidence exists to support weeds and other alternate crops serving as non-*Bt* refuge?

There are several plants ECB and CEW/CBW can survive on throughout the cornbelt and in the southern U.S. However, studies in central Iowa suggest that ECB production from alternate hosts is small and unreliable. It is expected that alternate host production of ECB and CEW/CBW will vary regionally. Producers should not rely on these sources for refuge until they are proven to be substantial and reliable.

G. Refuge structure--cotton:

1. Is there any scientific evidence to indicate whether the 20% sprayed or 4% unsprayed option is superior to the other?

No.

2. Is there any scientific evidence (field evidence) that either or both of EPA's mandated refuge options (20% sprayed or 4% unsprayed) are inadequate for long-term resistance management? What is this information?

The Subpanel stated that there is no field evidence at this time. The extension publication by Simmons *et al.*, (1998) was noted as an attempt to begin to develop field evidence for PBW in Arizona. This publication describes a multi-year, in-field study evaluating the efficacy of five *Bt* deployment strategies for management of resistance to CryIA(c) in PBW. The deployment strategies are: in-field refuges, rotation in years, *Bt* plus parasitic nematodes, external refuges, and 100% *Bt* cotton. It is anticipated that results from this study will become available subsequent to the 1999 growing season.

However, during the writing of this Subpanel Report, and consistent with other portions of this Report discussing the value of models, some members of the Subpanel noted that current EPA regulations are vague regarding the placement and effective size of refuges. Research on seasonal movement of TBW suggests that substantial local population substructure can develop during the summer as a result of restricted movement (Han and Caprio, unpublished data). PBW is also known to have limited movement during summer generations (see references in Gould and Tabashnik, 1998). Model simulation results have suggested that wild hosts and crops that are not attractive to heliothine moths continuously throughout the season are much less effective at managing resistance than temporally stable structured refuges. Furthermore, currently available *Bt* cotton does not produce a high dose for CBW/CEW. Until it is shown that non-cotton hosts produce enough susceptible moths to significantly delay the evolution of resistance in CBW/CEW populations exposed to moderate *Bt* doses, non-*Bt* cotton acreage must be considered the primary source of susceptible CBW/CEW moths. Based on these considerations, the Subpanel suggested that the EPA should reassess current resistance management strategies with regard to the distance between refuges and transgenic crops and the expected production of susceptible insects from different types of refuges.

3. Should refuge size vary depending on the targeted pest (i.e., TBW, CBW/CEW, or PBW)?

Perhaps, but this needs further evaluation.

Question 10: Monitoring

A. Does the Subpanel agree with EPA that surveillance and monitoring techniques should be tailored specifically for *Bt* potato, *Bt* corn, and *Bt* cotton?

The Subpanel agreed with this statement and considers surveillance and monitoring techniques essential. Because each targeted insect species possesses different life histories and feeding strategies, and because of vast differences in acreage of the three transgenic crops involved, each crop/insect combination will require much different sampling patterns as well as specific techniques for assessing *Bt* susceptibility.

B. Does the Subpanel agree that the collection of baseline susceptibility data for the targeted pests of *Bt* potato, *Bt* corn, and *Bt* cotton should be continued and the discriminating dose concentrations validated? What information is lacking regarding baseline susceptibility data and discriminating dose concentration development for the targeted pests of *Bt* potato, *Bt* corn, and *Bt* cotton?

The Subpanel agreed that collection of baseline data is important, and that it will provide information that is essential to managing resistance in pest populations, especially in assessment of whether a field control failure was due to actual resistance or other factors affecting expression of the *Bt* toxin. These baseline data also will be helpful in documenting the extent and distribution of resistance. If resistance develops, monitoring techniques will be required to test the effectiveness of resistance management programs designed to reduce the frequency of resistant individuals.

Information that is lacking includes:

(a) Reliable ways to measure resistance levels, especially for populations of insects that survive and damage transgenic plants in the field.

(b) Identification/validation of discriminating doses for monitoring specific pests. It may be advisable to establish procedures to monitor a range of doses for a single stage of the targeted pest (e.g., neonate larvae). Since first instars may be difficult to detect by growers, consultants, and extension personnel, additional monitoring techniques that utilize discriminating concentrations for later instars should be established.

(c) Development of simpler and more rapid field and laboratory bioassays.

(d) A diagnostic technique employing a single concentration, e.g., the LC99 of a susceptible population, which will be more sensitive to detecting resistance than an LC50 or LC90.

(e) Because of the limitations of bioassay-based monitoring, other techniques such as sentinel plots should be investigated. A possible advantage of these techniques is that they could screen large numbers of insects. Field-based approaches to monitoring probably will not replace diagnostic techniques. If suspicious insects are found in the field, they still must be brought to the laboratory so that resistance can be documented and quantified. The field methods might be useful and more sensitive than the diagnostic methods because they potentially screen many more individuals.

(f) The Subpanel recognized the need to evaluate large numbers of individuals from as many locations as possible. This might require collecting as many as 3,000-6,000 individuals from a location for a colony (e.g., Roush and Miller, 1986). Sample numbers as high as this, however, might not be practical. Sample sizes of 50 or less to initiate colonies may be more realistic. We may have to do the best we can with what we have, but we should continue to strive for as many parental targeted pests as possible.

C. Does the Subpanel agree with EPA's analysis that routine monitoring for *Bt* resistance to the Cry proteins for each of the targeted pests should be performed?

The Subpanel agreed with the principle of routine monitoring and believed it should be conducted annually for each Cry protein for each targeted pest. Without such an effort, baseline data that have already been established will have little or no value.

1. Discuss whether triggered monitoring, rather than routine monitoring, in high density *Bt* crop sites is appropriate.

Based on the innate variability in insect responses to *Bt* toxins, it is essential to use a multi-component approach to monitoring. Ideally, this system would consist of annual sampling in areas where transgenic plants have the highest market share ("triggered" or "tiered" monitoring). Additional samples from other areas where transgenic plants have not impacted the market to the same degree would also be important for comparative purposes ("routine" monitoring). Finally, development of a field-surveillance approach that increases the sensitivity of resistance detection also could be employed, especially in areas of highest market penetration. This multi-component approach to monitoring should be tailored for the individual crop and production areas and should be determined by regional working groups.

2. Should routine monitoring be performed at random or set sites?

Attempting to cover the entire distribution of the insect being monitored at the expense of more detailed monitoring in areas of high density of *Bt* crops is not recommended. The Subpanel preferred a combination of both, but the degree of each will depend on the insect/crop involved and should be determined separately for each situation. If there is relative homogeneity in crop production within a region, random sampling would be adequate. Where there are large differences in concentration of *Bt* crops within a region, samples should be intensified in those areas of high *Bt* acreage.

D. Does the Subpanel agree with EPA's analysis that current monitoring programs for CPB, ECB, CEW/CBW, TBW and PBW are adequate? Is there any scientific evidence to suggest that these monitoring programs should be altered?

If the goal is to detect subtle changes in frequency of resistance alleles prior to a control failure, then the answer is no. Therefore, it is recommended that other approaches to monitoring that may increase sensitivity (e.g., sentinel plots) should be considered. However, because of resource limitations, we have to do the best we can with what we have. From this standpoint, the answer is yes. Existing programs should be maintained and used to identify whether control failures are related to resistance or other factors affecting expression of the toxin. If resistance occurs in the field, extensive monitoring will be needed and should be increased substantially to determine stability, cause, and geographical extent of resistance.

E. What types of information or education are necessary to monitor for the key pests in a crop system? Given these requirements, is it feasible for an average grower to adequately monitor for key pests?

"Monitoring" for key pests in a crop system is entirely different from "resistance monitoring". Growers (and/or their pest advisors, crop consultants, extension personnel, etc.) are in most cases capable of monitoring pest species and their associated damage in both *Bt* and conventional crops. All stakeholders should be taught the importance of monitoring, along with practical considerations, such as accurate planting records. They also should be considered partners in managing insect resistance.

Question 11: Remedial Action Plans

Registrants have reported to EPA incidences of control failures which might have been attributed to resistance. These incidences were investigated by the registrants. No incidences were found to be related to the development of insect resistance in any of the targeted pests of *Bt* potato, *Bt* corn, or *Bt* cotton.

A. Remedial action plans:

1. Does the Subpanel agree with EPA's analysis that current remedial action plans for *Bt* potato, *Bt* corn, and *Bt* cotton are adequate? If there are other remedial actions not included in the current plans, what are these and what personnel would be needed to implement these actions?

The current remedial plans are a necessary starting place, but they were not deemed fully adequate (and perhaps cannot be) prior to gaining knowledge that will only come once resistance is detected in field populations. EPA has outlined steps for *Bt* corn, *Bt* potato, and *Bt* cotton that are sensible: required reporting of resistance events to the Agency within 30 days, notification within 90 days of mitigation measures, working with the Agency to implement long-term resistance management, etc. However, what is missing from the remedial action plans are pivotal details that could greatly influence resistance management but that will need to be determined on a regional basis. For example, how likely are we to detect resistance to *Bt* in different production areas? Who decides what constitutes workable monitoring plans? Should not the adequacy of *Bt* monitoring be discussed on a regional basis with relevant University and USDA experts? Is monitoring being done on a scale that is likely to detect resistance events in isolated or distinct production areas of the three commodities? Are we going to trigger remedial action based on small changes in susceptibility to *Bt* of field populations? Which remedial actions are most likely to succeed in a given crop and region?

While it is necessary for EPA to require remedial action if resistance is confirmed, it is obvious that great uncertainty will be involved in discerning the significance of resistance in its early stages of development, especially if minor mechanisms evolve. This was noted by Hardee and Adams (cited by EPA, 1998, p. 66) who stated that "Determination of threshold levels of initiating remedial action need to be developed as well as the specific programs for appropriate remedial action". For cotton, remedial actions suggested by the Agency include informing customers and extension agents in affected areas, implementing alternative means to control resistant populations, increasing monitoring in affected areas, and ceasing of sales in affected and bordering counties (EPA, 1998, p. 66). Again, the significance of regional input into such plans is clear. In states with isolated production areas and very large counties, it would be illogical and punitive to mandate that sales in bordering counties cease in the absence of field data indicating that this is needed.

In conclusion, the remedial action plans devised by EPA provide a framework for further refinement. Regional working groups of experts should be convened to modify and refine them to specific production settings. The Subpanel recommended that the current remedial action plans be further defined and refined on a regional- and crop-specific basis. Specific recommendations in this regard are that:

(a) Regional *Bt* working groups be involved in designing and overseeing pro-active programs to monitor in a coordinated and systematic fashion, efficacy of *Bt* transgenic plants in the field, resistance of key pests in bioassays, and identification of mechanisms for funding such monitoring;

(b) Exchange of data on the development of resistance to *Bt* crops be fostered by the regional working groups convened by EPA on an annual (or when deemed necessary) timetable;

(c) EPA should continue to: (i) foster producer education programs focusing on detection and management of resistance to *Bt* crops and the critical need for complying with resistance management strategies; and (ii) promote rapid dissemination of information to producers and pest managers.

2. If resistance is suspected, what data should be collected at the sites reporting crop failures?

The Subpanel felt that the Agency (EPA, 1998, pp. 66-68) adequately detailed needs in this area. Data collection should focus on the following:

(a) Sample pest density at the location of the putative resistant population to confirm that it has reached densities deemed unusual for that *Bt* crop;

(b) Sample plants at the location of the putative resistant population to confirm that they are expressing the *Bt* toxin;

(c) Sample pest density in other fields of *Bt* crops within the immediate vicinity to define the extent of the problem;

(d) Place the suspect population into culture and bioassay it for susceptibility to the appropriate *Bt* toxin;

(e) If resistance is confirmed by laboratory bioassays, then cease using *Bt* in the problem area and conduct studies on the rate of decline of resistance in the field;

(f) Determine cross resistance relationships of resistant population to other *Bt* toxins.

Only if regional programs with adequate action are underway to monitor the efficacy of *Bt* transgenic crops will facilities and expertise be in place to accomplish these objectives in a timely fashion and to rapidly disseminate results to users of transgenic corn, cotton and potato.

B. 1996 Brazos Valley incidents:

1. In terms of the reports of *Bt* cotton performance failures in the Brazos Valley, Texas, and other areas in 1996, does the Subpanel agree with EPA's analysis that resistance was not the cause of the 1996 bollworm outbreak?

Yes.

2. What other data, if any, should have been collected as part of the investigation of these incidents?

The Subpanel felt that investigations into this situation were sufficient. No suggestions for additional data collection were offered.

3. What methods exist to improve the reporting, analysis, and action taken in response to this type of incident?

The question of appropriately responding to the Brazos Valley incidents is a specific case of the more general question (see response to Question 11A2 above) of devising and implementing appropriate plans for responding to putative resistance events.

Question 12: Resistance Management Considerations for Other Cropping Systems.

A. Are refuges necessary to manage resistance on crops planted on limited acreage (i.e., crops under 300,000 acres)? What factors should EPA consider in evaluating the adequacy of resistance management for such crops? For example, over 90% of the artichoke crop (grown only in limited areas in California) is treated with *Bt* microbial pesticides. If a *Bt* artichoke was developed, would a refuge be important?

The Subpanel recognized the importance of targeting resistance management at pest populations. Unfortunately, population structure of most pest populations is not clearly understood and the relative contributions of different crops and wild hosts to overall population size are generally not available. Unless there are data indicating that there are sufficient refuges for the pests in other crops and in native vegetation, the Subpanel recommended that all *Bt* crops include a structured refuge. Further, foliar *Bt* products should not be used in the refuge created to produce *Bt* susceptible insects.

On some vegetable crops, the transplant industry is important and often serves as a source of resistance development for various pest species. The concept of a structured refuge should include the transplant and glasshouse industry in these cases.

Refuge areas should be carefully managed to insure that susceptible insects survive. Overuse of insecticides that significantly reduce numbers of susceptible insects in the refuge areas should be avoided.

B. What additional resistance management elements might be needed for Cry proteins expressed in vegetable and fruit (tree, vine, etc.) crops? What might be eliminated as unnecessary?

Resistance management considerations and elements in vegetable and fruit crops should be similar to those discussed for *Bt* corn, *Bt* cotton and *Bt* potato. Further, foliar *Bt* products should not be used in the refuge created to produce *Bt* susceptible insects.

The Subpanel recognized the value of regional considerations in this process. A total ecosystem approach considering important economic impacts is important.

1. What are the resistance management considerations for *Bt* tomato including the use of a refuge? How will the use of *Bt* tomato affect *Bt* microbial pesticides in tomato or other crops?

Considerations for *Bt* tomato and other vegetable crops should be similar to those described for *Bt* corn, *Bt* cotton and *Bt* potato.

2. What are the resistance management considerations for *Bt* delta endotoxins expressed in minor crops such as broccoli, including refuge, to control such pests as the DBM and the cabbage looper? How will these uses affect *Bt* microbial pesticides in broccoli or other crops?

They would be the same as those described for *Bt* corn, *Bt* cotton and *Bt* potato.

3. What are the resistance management considerations for *Bt* canola, including refuge?

Again, they are the same as those described for *Bt* corn, *Bt* cotton and *Bt* potato.

C. What are the resistance management considerations, including refuge, for *Bt* delta endotoxins used in forestry uses, such as *Bt* poplar harvested after 4 to 6 years? How will these uses affect *Bt* microbial pesticides in forestry uses?

Issues of scale will likely be important in forestry. Considerations of foliar *Bt* applications and the planting of *Bt* trees should be jointly valued in resistance management plans. Options in changing plantings of *Bt* trees are more restricted than with annual crops. However, the general factors important in resistance and the need for refuge areas are similar to those for *Bt* cotton, *Bt* corn and *Bt* potato. If resistance is detected, remedial efforts should be implemented to confine the population. Research addressing death of susceptible trees in refuges needs to be conducted to determine whether replacement of dead non-transgenic trees with similar but different age stock several months to years after the planting was established, affects refuge value (implications for production of susceptible pest genotypes, etc.).

Question 13: Grower Acceptance

A. For *Bt* cotton, EPA has mandated a 20% sprayed or 4% unsprayed refuge. Based on surveys (conducted by Monsanto), more growers implemented the 20% sprayed refuge option over the 4% unsprayed refuge option. What are the various factors growers considered in choosing the 20% or 4% refuge option?

Simple economics of the impact of insects on the unsprayed refuge drove widespread choice of the 20% option in several areas of the southern cotton belt. In contrast, in Alabama and a few other areas, the threat of TBW resistance to pyrethroids and associated risk perceptions of bankers drove widespread acceptance of the 4% option. As a result, market penetration of Bollgard cotton in some areas of Alabama reached nearly 77% in 1996 and 70% in 1997 with the slight drop reflecting greater grower acceptance of the 20% option (Monsanto Company, 1997). Popularity of the 20% option may reflect a desire to protect refuge cotton with foliar insecticides. This may or may not be the best option for resistance management. Growers prefer having different options and the ability to tailor decisions to their individual production situation. For example, in Arizona, widespread variability in acceptance of *Bt* cotton (30 to 80% in different regions) reflects differences in production systems and risk of pest losses. As discussed earlier, the severe risk of PBW losses in some areas may limit economic willingness to accept these losses on a 4% unsprayed refuge. As growers attempt to integrate *Bt* cotton into IPM systems, the presence of stacked genes for herbicide resistance could limit production options and interfere with establishment of in-field refuges.

B. For *Bt* potato/*Bt* corn, EPA has not mandated specific refuge options. Based on grower surveys and personal experience, what factors do growers consider to be important regarding the implementation of refuge?

This is addressed in the context of an earlier consensus that a structured refuge, mandated or voluntary, needs to be established for both *Bt* corn and *Bt* potato and that adoption of a structured refuge is critical to success of a high dose/refuge approach to resistance management. Consequently, refuge options, whether mandated or voluntary, need to reflect economic, logistical, and sociological dimensions of grower adoption as well as the ecological realities of the pest/crop system. Key production and biological factors affecting resistance management in potato are discussed by Kennedy and Whalon (1995). These factors, which also generally apply to other *Bt* crops, include: (a) *Bt* crop efficacy and yield performance, (b) personal risk, (c) corporate risk, (d) voluntary or mandatory option(s), (e) economics, (f) ease-of-implementation, (g) land management, (h) past history of resistance, (i) alternative controls and their efficacy, (j) implementation support (training, monitoring, consultants), (k) incentives (taxes, insurance, discounts), (l) IPM experience, and (m) attitudes of other growers.

Education is the key to adoption of resistance management strategies, whether mandatory or voluntary. Growers need to know why a refuge is critical to resistance management. Surveys indicate that corn growers are receptive to education about this new technology, including issues related to resistance management. A 1995 Iowa survey (Pilcher and Rice, pers. comm.) found that 83% of farmers would be willing to implement some form of resistance management, if the plan was recommended by seed companies or universities. Similarly, a 1996 survey of Illinois, Iowa, Kansas, Minnesota, Nebraska, and Pennsylvania growers of *Bt* corn (Event 176) (Rice *et al.*, unpublished) found widespread willingness (>79%) to adopt a compatible resistance management strategy. Over 73% of respondents were willing to plant 25% or greater refuge. However, widespread yield advantages of 15-40 bushels per acre associated with *Bt* corn production in the western corn belt (western Minnesota, South Dakota, Nebraska, and western Iowa) in 1997 have fueled high demand for *Bt* corn and may have markedly changed willingness to leave an unmanaged refuge. Bankers have even begun requiring farmers to plant *Bt* corn as an insurance against the risk of crop loss.

Regional variation in production systems, pest ecology and risk of insect losses support flexibility in the refuge component of resistance management plans. Practical issues related to implementation are crucial to success of the refuge. These include logistical considerations in planting, insecticide treatments and harvesting procedures. Non-targeted pests also need to be considered in the development of refuge recommendations (e.g., SWCB in Kansas, Texas and Oklahoma; CEW/CBW in southern and midwestern corn).

Stacked genes, especially those that confer herbicide resistance, may complicate management decisions regarding in-field refuges. Efforts by seed companies to reduce problems with maturity and gene stacks (e.g., herbicide resistance) in hybrid pairings, provide identification of varieties with higher native tolerance or resistance, and increased technical support for managing insect pests in the refuge would enhance grower acceptance and implementation of refuges.

An incentive-based refuge concept may encourage higher levels of adoption and will be better received by growers than the mandated, punitive-based concept evident in some current grower agreements. Associated educational efforts that explain why resistance management is important combined with a consistent message from university extension, the seed industry, and farm advisors would enhance grower adoption.

III. RESEARCH NEEDED TO IMPROVE ABILITY TO IMPLEMENT RESISTANCE MANAGEMENT

To be effective in managing resistance, refuges must produce large numbers of adults relative to the number produced in transgenic crop areas. The Subpanel suggested that a production of 500 adults in the refuge that move into the transgenic fields for every adult in the transgenic crop area (assuming a resistance allele frequency of $5x10^{-2}$) would be a suitable goal. (See Attachment I of this Report). A resistance management program should continue to provide benefit until the resistance allele frequency is at least this high. In order to accomplish this goal, further research is needed to provide detailed knowledge of pest population dynamics in treated and untreated refuges, as well as alternative hosts (if those are assumed to be acting as refuges). In addition, the impact of sprays and IPM practices on the efficiency of refuges (numbers of adults produced) must be a research priority.

The placement and size of refuge patches is critical and can impact the effectiveness of refuges. Growing non-*Bt* host plants in the immediate vicinity of transgenic plants is likely to decrease the degree of non-random mating between individuals produced in refuges and transgenic crops. However, creating refuge patches that are too small may result in much of the reproductive effort of refuge individuals being spent in transgenic areas (essentially suicidal for susceptibles) and a reduction in effective refuge size. Research is needed to understand when and where females mate (female movement and behavior), as well as the gene pool they are likely to mate with which is strongly affected by male movement. Information is also needed on ovipositional patterns, where females lay eggs in relation to transgenic and non-transgenic plants.

Recruitment patterns into and out of refuges at the start and end of each season, or between crops within season, are important determinants of refuge effectiveness. If refuges are semi-isolated, such that refuge and transgenic patches are not in panmixia, it is expected that refuges will contain a larger proportion of the population than one would expect from area alone. If recruitment into a new crop is random across the two habitats, the ratio of adults in refuges compared to transgenic areas would be decreased and the efficiency of the refuges diminished. Further information is also needed on overwintering survival under various host plants, as well as the variability in this parameter. Finally, the impact of cultural practices, including tillage and weed management, on overwintering patterns and recruitment is poorly understood.

There are additional implementation issues for refuges that can impact the evolution of resistance and the acceptance of refuges in the cropping systems. These include improving the integration of a refuge strategy with IPM practices. Research also is needed to determine the optimal trade-offs among logistic, sociological, economic, and biological concerns. The best refuge strategy from a biological standpoint may not be effective if not acceptable because of logistic, economic, or sociological concerns.

More research is needed on the interactions of biological control agents and alternative control strategies on the efficiency of refuges and on the impact of transgenic crops on the population dynamics of biological control agents in general. If resistance does occur, are there potential lag times between the resurgence of pest populations and biological control agents, and if so, are there potential refuge designs that might reduce that lag time?

Further research is needed to determine if there are potential methods to predict cross-resistance patterns. This would include continued selection of colonies resistant to *Bt* endotoxins. Some comparisons of resistant DBM and TBW suggest that there is a potential to develop some generalities.

The collection and storage of DNA samples in conjunction with resistance monitoring should be encouraged. Once resistance has developed, efforts should be made to develop markers for resistance genes and utilize stored genetic material to provide a much improved retrospective analysis of the evolution of resistance. This would improve resistance management strategies in the future and assist in evaluation of the effectiveness of the present strategies.

Research addressing larval movement and impact on fitness is needed. The high dose/refuge strategy requires that insects either be exposed to no toxin or be exposed to a dose of toxin that is 25 times the dose needed to kill 99% of susceptible insects. The high dose is intended to reduce survival of insects with partial resistance to the toxin. If fields are planted to a mixture of *Bt* producing plants and non-*Bt* plants (i.e., seed mix), there is potential for larvae to move from plant to plant. If a larva feeds on a *Bt* plant for a short period of time then moves to a non-*Bt* plant, it may receive an average dose of toxin below the high dose level. This would presumably allow survival of partially resistant larvae. Larval movement from non-*Bt* to *Bt* plants in late larval instars could have the same effect. Until more is known about the movement patterns of a number of targeted pests, it will be difficult to know if a seed mixture could be detrimental to the high dose/refuge approach. Research on larval movement during the entire developmental period of lepidopteran pests is needed. (For the CPB, data are also needed on movement of adults because they feed on the crop plants). Research on the fitness of individuals that move from one plant type to another at different developmental stages is also needed. If fields are planted as row by row mixtures, movement could also be detrimental to the high dose approach, so similar research should be conducted on these row by row mixtures.

Studies on initial resistance allele frequencies must be continued. Research aimed at assessing the frequency of major resistance alleles in field populations of targeted pests has recently begun. At least 3 distinct, useful approaches have been developed, and it is possible that other approaches will soon be developed. Although knowing the initial frequency of resistance alleles in every population of every targeted pest cannot be justified, it is important to have a general understanding of what the initial frequencies of major resistance alleles are as the high dose/refuge approach is implemented. As indicated in the body of this Report, the size of refuges needed to make the high dose/refuge approach work is related to initial resistance allele frequency. At this point these estimates do not exist for all targeted pests and therefore the few preliminary estimates available must be relied on. It would be very useful to have at least one good estimate of initial frequency for each targeted pest species.

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Attachment I

EXPLANATION OF HOW THE SUBPANEL ARRIVED AT 500 SUSCEPTIBLE PER ONE RESISTANT INSECT FOR EFFECTIVE RESISTANCE MANAGEMENT

Ratio of Susceptible: Resistant Insects Needed to Delay Resistance Development

OBJECTIVE:

Determine a reasonable ratio of resistant insects produced from *Bt* crops to all insects produced in the refuge (that are available for mating) that would significantly slow the evolution of resistant pest populations.

METHODS:

Simple Model

A single locus, two-allele model which assumes random mating (Gould, 1994) was used to determine how many total insects would have to be produced in a refuge compared to the number of resistant insects (must have one or more resistance alleles) produced in the *Bt* crop. Most of the simulations assumed that there was a high dose that made resistance nearly recessive. It was assumed that the fitness of all genotypes on the non-*Bt* hosts was 1.0. The fitness of SS and RR individuals on *Bt* plants was always held at 0.001 and 1.0, respectively. The fitness of RS individuals on *Bt* plants varied (0.002, 0.004, 0.008,0.016, 0.032, 0.500). To get ratios of insects from the refuge and *Bt* crop that ranged from 200:1 to 1715:1 with the different assumptions about RS survival, the effective size of the refuge (Gould, 1998) was varied from 4% to 50%. All ratios were computed at allelic frequencies of 0.005. For each combination of refuge size and RS fitness, the model was run with the assumption of initial allelic frequency of 0.001 and 0.005.

> ***Results*:** Results from all of the runs of the model are presented in the Table. When RS fitness of *Bt* plants was set at 0.002, a ratio of 930:1 was obtained. If the initial R allelic frequency was 0.001, it took 36 generations for the R allelic frequency to reach 0.50. If the insect pest had 2 generations per year, the 4% effective refuge that resulted in the 930:1 ratio would give about 18 years of protection. If the initial allelic frequency was 0.005, this time was reduced to about 7 years. As RS fitness was increased, the insect ratio decreased and adaptation took less than 10 years, even with a 0.001 initial frequency.
>
> When effective refuge size was increased to 10%, a ratio of 257:1 was established, even when the RS fitness on *Bt* crops was 0.032. With these assumptions it took 19 generations for the allelic frequency to change from 0.001 to more than 0.50.

One model run was executed using a RS fitness of 0.5 on *Bt* plants. This run simulates what would happen if the high dose was not achieved and there was high RS fitness. In this case, a 50% effective refuge gave a ratio of 200:1, and with this large refuge it took 19 generations for the allelic frequency to rise from 0.001 to more than 0.50.

***Conclusions*:** A specific ratio of all refuge insects to resistant insects produced in the *Bt* crop is not a perfect indicator of the relative effectiveness of a resistance management plan, but it does give a fairly robust estimate of effectiveness over a diverse set of genetic and environmental conditions. **A good resistance management strategy should provide efficacy of the toxin(s) for more than 10 years. Therefore, a ratio of less than 500:1 may lead to resistance in less than 20 generations and therefore in less than 10 years because many of the targeted pests have 2 to 4 generations exposed to *Bt* plants each year.** This would translate to less than 10 years of effectiveness (under the stated assumptions). The simple model assumes the same ratio throughout the season. Since this is unlikely to be the case, it is therefore necessary to examine a portion of the genetic and environmental parameters using a more complex model that includes population dynamics and the possibility of non-random mating and oviposition.

<u>Complex Model</u>

The complex model assumed survivorship rates of 4, 8, and 95% for the SS, RS, and RR individuals, respectively. Overwintering occurred every 130 days in the model (the insects were either in alternative crop/wild host systems or in diapause for the remainder of the year). The simulated universe consisted of 400 habitat patches, 360 of which were cotton habitat and 40 were wild host habitat. Fifteen percent of the population in a cotton habitat patch dispersed to another patch each day (the new patch could also be cotton habitat), while 20% of the wild host population migrated per day. Females mated on the first day after emergence and again 5 days later. In the simulations, 85% of the females in transgenic crops therefore mated prior to dispersal. The male population these females mated with, however, would have been more representative of the overall genetic composition of the population. Two levels (90% and 30%) of overwintering dispersal were simulated. At the 30% level, most individuals that were in a refuge in one year returned to that refuge at the start of the following year, while at 90% dispersal level, recruitment into two different habitat types in the spring was more random. The lower the overwintering dispersal level, the greater the bias of the spring time recruitment into refuges compared to recruitment into transgenic patches.

***Results*:** The model was run without simulating the presence of the *Bt* endotoxins for the first year. The refuge populations under these circumstances accounted for slightly less than 10% of the total population (see Figure). The time to resistance was strongly affected by the overwintering dispersal, and the time taken for resistance to evolve with a low overwintering dispersal rate was 4-fold greater than with a high overwintering dispersal rate was simulated. The primary effect of this difference in overwintering dispersal is the relative size of the refuge immediately after overwintering (see Figure). At the end of a season, a large portion of the population resides in refuges. When overwintering dispersal is high, few of these individuals are recruited back into the refuges in the following year. When the overwintering dispersal is low, the early season refuges have a high recruitment level from the previous season's refuge populations.

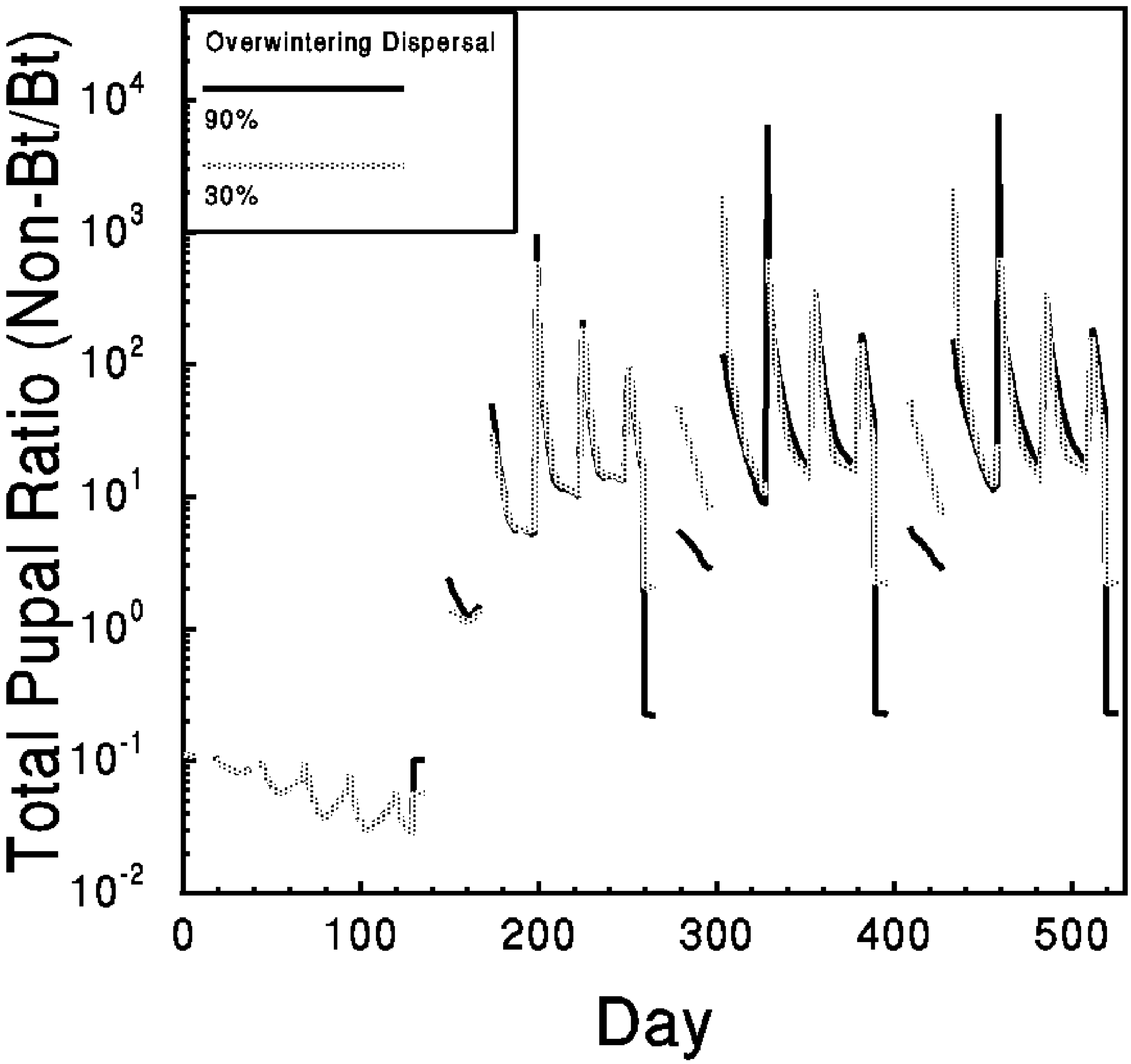
Conclusions**:** The ratio of non-*Bt* to *Bt* field populations varied from 0.3 to more than 10,000 over the course of a single season (see Figure). Overall, the arithmetic mean ratio of pupae in the refuges compared to the *Bt* fields was 77.66 and 83.99 for the high and low dispersal rates, respectively. The harmonic means for these dispersal rates were 2.91 and 15.38 The ratio of the harmonic means (5.27) was closer to the ratio of time to resistance (4.35) than the ratio of the arithmetic means (1.08) or the ratios of the geometric means (1.43). These results tentatively suggest that the harmonic mean (which emphasizes the lowest ratios over time) may be a better estimate than the arithmetic or geometric means. Based on Hardy-Weinberg expectations and the survivorship ratios of the three genotypes to the transgenic crop, 92% of the survivors in the transgenic fields were homozygous susceptible individuals. The ratio of the refuge population to the resistant population (individuals of the RS or RR genotypes) in transgenic fields was much greater than the ratio of total populations. For the high dispersal simulation, the corrected arithmetic and harmonic means were 970.75 and 36.4, while the low dispersal means were 1049.9 and 192.2. The corrected means, though more difficult to estimate in field populations, are less dependent on the fitness of susceptible individuals and should be used when possible.

TABLE

Estimating how the ratio of all insects in the refuge to resistant insects in the *Bt* fields impacts the rate at which resistance spreads within a randomly mating population. Fitness of the RR individuals on *Bt* plants is always assumed to be 1.0 and fitness of SS individuals on *Bt* plants is always assumed to be 0.001. Ratios are always computed for a resistance allele frequency of 0.005.

% Refuge	RS Fitness	Initial R Frequency	Ratio at R=0.005	Generations Until R=0.50
4% 4%	0.002 0.002	0.001 0.005	930:1 930:1	36 13
4% 4%	0.004 0.004	0.001 0.005	645:1 645:1	26 12
4% 4%	0.008 0.008	0.001 0.005	398:1 398:1	19 10
10% 10%	0.004 0.004	0.001 0.005	1715:1 1715:1	59 23
10% 10%	0.008 0.008	0.001 0.005	1063:1 1063:1	40 20
10% 10%	0.016 0.016	0.001 0.005	604:1 604:1	27 16
10% 10%	0.032 0.032	0.001 0.005	257:1 257:1	19 12
50% 50%	0.500 0.500	0.001 0.005	200:1 200:1	19 15

FIGURE


Overwintering Dispersal
90%
30%
Total Pupal Ratio (Non-Bt/Bt)
Day
0
100
200
300
400
500

Attachment II

LIST OF COMMON AND SCIENTIFIC NAMES AND ACRONYMS FOR PESTS OF CORN, COTTON AND POTATO

CORN:

Common Name	Scientific Name	Acronym
beet armyworm	*Spodoptera exigua* (Hübner)	
common stalk borer	*Papaipema nebris* (Guenée)	
corn earworm	*Helicoverpa zea* (Boddie)	CEW/CBW
corn leaf aphid	*Rhopalosiphum maidis* (Fitch)	
cutworms		
black cutworm	*Agrotis ipsilon* (Hufnagel)	
dingy cutworm	*Feltia jaculifera* (Guenée)	
other cutworms	Family Noctuidae	
European corn borer	*Ostrinia nubilalis* (Hübner)	ECB
fall armyworm	*Spodoptera frugiperda* (J. E. Smith)	
hop vine borer	*Hydroecia immanis* Guenée	
potato stem borer	*Hydroecia micacea* (Esper)	
rootworm complex		
banded cucumber beetle	*Diabrotica balteata* (LeConte)	
northern corn rootworm	*D. barberi* (Smith & Lawrence)	
spotted cucumber beetle	*D. undecimpunctata howardi* (Barber)	
western cucumber beetle	*D. undecimpunctata undecimpunctata* (Mannerheim)	
western corn rootworm	*D. virgifera virgifera* LeConte	
Mexican corn rootworm	*D. virgifera zeae* Krysan & Smith	
sod webworms	Subfamily Cramdinae	
southern corn stalk borer	*Diatraea crambidoides* (Grote)	
southwestern corn borer	*Diatraea grandiosella* Dyar	SWCB
spider mites	*Tetranychus* spp.	
true armyworm	*Pseudaletia unipuncta* (Haworth)	
western bean cutworm	*Richia albicosta* (Smith)	

COTTON:

Common Name	Scientific Name	Acronym
beet armyworm	*Spodoptera exigua* (Hübner)	
boll weevil	*Anthonomus grandis grandis* (Boheman)	
cabbage looper	*Trichoplusia ni* (Hübner)	
cotton aphid	*Aphis gossypii* Glover	
cotton bollworm	*Helicoverpa zea* (Boddie)	CBW/CEW

cotton fleahopper	*Pseudatomoscelis seriatus* (Reuter)	
cotton leaf perforator	*Buccalatrix thurberiella* Busck	
cotton square borer	*Strymon melinus* Hübner	
European corn borer	*Ostrinia nubilalis* (Hübner)	ECB
fall armyworm	*Spodoptera frugiperda* (J. E. Smith)	
Lygus bug	*Lygus hesperus* Knight	
pink bollworm	*Pectinophora gossypiella* (Saunders)	PBW
plant bug complex	Family Miridae	
saltmarsh caterpillar	*Estigmene acrea* (Drury)	
southern armyworm	*Spodoptera eridania* (Stoll)	
soybean looper	*Pseudoplusia includens* (Walker)	
spider mites	*Tetranychus* spp.	
stink bug complex		
brown stink bug	*Euschistus servus* (Say)	
green stink bug	*Acrosternum hilare* (Say)	
southern green stink bug	*Nezara viridula* (Linnaeus)	
tarnished plant bug	*Lygus lineolaris* (Palisot de Beauvois)	
tobacco budworm	*Heliothis virescens* (Fabricius)	TBW
whitefly complex		
bandedwinged whitefly	*Trialeurodes abutiloneus* (Haldeman)	
silverleaf whitefly	*Bemisia argentifolii* (Bellows & Perring)	
sweetpotato whitefly	*Bemisia tabaci* (Gennadius)	

POTATO:

Common Name	Scientific Name	Acronym
aphids	Family Aphididae	
aster leafhopper	*Macrosteles quadrilineatus* Forbes	
blister beetles	Family Meloidae	
Colorado potato beetle	*Leptinotarsa decemlineata* (Say)	CPB
cucumber beetles	*Diabrotica* spp.	
European corn borer	*Ostrinia nubilalis* (Hübner)	ECB
flea beetles	Family Chrysomelidae	
garden symphylan	*Scutigerella immaculata* (Newport)	
grasshopper	Family Acrididae, primarily *Melanoplus* spp.	
leaf miners	*Liriomyza* spp.	
onion thrips	*Thrips tabaci* Lindeman	
potato leafhopper	*Empoasca fabae* (Harris)	
potato psyllid	*Paratioza cockerelli* Sule	
potato tuberworm	*Phthorimaea operculella* (Zeller)	
spider mites	*Tetranychus* spp.	
threelined potato beetle	*Lema trilinea* White	
wireworms	Family Elateridae	

OTHER:

Common Name	Scientific Name	Acronym
diamondback moth	*Plutella xylostella* (Linnaeus)	DBM

FOR THE CHAIRMAN:

Certified as an accurate report of findings:

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Elizabeth Milewski, Ph.D.
Designated Federal Official
FIFRA Scientific Advisory Panel

Date: ______________________________