October 18-20, 2000 FIFRA SAP Meeting:
Bt Plant Pesticides Risk and Benefits Assessment

Background for Insect Resistance Management Questions

The following list will assist the reader with the acronyms for the insect pests discussed in these questions:

Acronym	**Common Name**	**Scientific Name**	**Crop**
BCW	Black Cutworm	*Agrotis ipsilon* (Hufnagel)	corn
CBW	Cotton Bollworm	*Helicoverpa zea* (Boddie)	cotton
CEW	Corn Ear Worm	*Helicoverpa zea* (Boddie)	corn
CPB	Colorado Potato Beetle	*Leptinotarsa decemlineata* (Say)	potato
CSB	Common Stalk Borer	*Papaipema nebris* (Guen.)	corn
ECB	European Corn Borer	*Ostrinia nubilalis* (Huebner)	corn
FAW	Fall Armyworm	*Spodoptera frugiperda* (J. E. Smith)	corn
PBW	Pink Bollworm	*Pectinophora gossypiella* (Saunders)	cotton
SCSB	Southern Corn Stalk Borer	*Diatraea crambidoides* (Grote)	corn
SWCB	Southwestern Corn Borer	*Diatraea grandiosella* (Dyar)	corn
TBW	Tobacco Budworm	*Heliothis virescens* (Fabricius)	cotton

<u>Question 1.</u> Definition of High Dose

Caprio et al. (1999) has suggested a 50-fold value be adopted (rather than 25-fold) because current empirical data suggest that a 25-fold dose may not be consistently high enough to cause high mortality among heterozygotes with known Bt resistance alleles.

The 1998 SAP Subpanel defined a high dose as "25 times the toxin concentration necessary to kill susceptible larvae." The Subpanel indicated that there were at least five imperfect ways to assess this 25-fold level, and that some approaches were more appropriate for specific crop pests. A cultivar could be considered to provide a high dose if verified by at least two of the following five approaches: (1) Serial dilution bioassay with artificial diet containing lyophilized tissues of Bt plants using tissues from non-Bt plants 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 in the field to make sure that the cultivar is at the $LD_{99.9}$ or higher to assure that 95% of heterozygotes would be killed (see Andow and Hutchison, 1998); (4) Similar to (3) above, but would use controlled infestation with a laboratory strain of the pest that had an LD_{50} value similar to field strains; and (5) Determine if a later larval instar of the targeted pest could be found with an LD_{50} that was about 25-fold higher than that of the neonate larvae. If so, the stage could be tested on the Bt crop plants to determine if 95% or more of the later stage larvae were killed. The Agency has used the 25-fold definition for high dose (and the five assessment techniques) since the 1998 SAP Subpanel meeting.

Bt Cotton

Question 2 through 7.

Refuge distance requirements and minimization of treatment of the refuge will increase the likelihood of success for the high dose/refuge strategy for insect resistance management in Bt cotton. Increasing the width of the refuge will increase the likelihood that susceptible adult females will lay at least some of their eggs within the refuge and not within the Bt cotton fields (a "source-sink" effect). Resistance risk can be decreased if the width is increased.

The 1998 SAP Subpanel noted that research has shown that substantial local population substructure can develop during the summer as a result of restricted movement of TBW and therefore, deployment of a refuge is important (SAP 1998). Because of this, Gould and Tabashnik (1998) recommended that the maximum distance between Bt cotton fields and the non-Bt cotton refuge should be less than or equal to one mile.

Based on ovipositional patterns for CBW, Caprio (2000a) has indicated that untreated embedded refuges should be at least 100 meters wide to minimize the risk of rapid resistance evolution associated with source-sink dynamics (i.e., the refuge must be wide enough so that all females do not lay all of their eggs in the Bt portion of a field and close enough to the Bt portion of the field so that there can be random mating and random oviposition of adults).

Preliminary data provided by Gould (see EPA, 1999) indicate that CBW are capable of moving from the north to the south. This type of movement may increase the resistance risk especially in cotton-growing areas.

PBW larvae movement is limited between plants, especially in reproductive cotton. Based on PBW dispersal information, in-field refuges or refuges placed as close to the Bt cotton fields as possible (i.e., <0.6 miles – see Tabashnik et al. (1999)) should increase the likelihood of the desired random mating between resistant and susceptible PBW adults.

Adamczyk et al. (2000) found that current Bt cotton varieties express different levels of Cry1Ac endotoxin throughout the plant and that reproductive isolation of populations of intrinsically tolerant Lepidoptera (CBW and fall armyworm) may occur and complicate the refuge strategies even further.

A summary of the existing refuge scenarios are provided in the Table below. Gould's and Caprio's models for TBW and CBW resistance management are described in the Agency's draft risk assessment document in Section D. and the two background Bt cotton IRM documents, also Appendix 1.

Summary of Refuge Scenarios for TBW, CBW, PBW Resistance Management

Refuge Scenarios	**External Unsprayed (Structured)**	**Embedded**	**External Sprayed**
TBW, CBW, and PBW: Required refuge for 2001 growing season * Seed growers must plant the refuge within 1 mile of the Bollgard cotton and as close as possible to Bt cotton fields when there is a conflict with seed production regulations	5% external unsprayed (150 ft. wide); planted within ½ mile	5% embedded (sprayable) - at least 150ft. wide (approx. 50 rows); For small or irregularly shaped fields, neighboring fields farmed by the same grower can be grouped into blocks to represent a larger field unit, provided the block exists within one mile squared of the Bollgard cotton and is at least 150 ft. wide. The refuge may be treated as long as the whole field(s) – Bt and non-Bt are treated. For PBW only, the refuge cotton may be planted as single rows within the Bollgard field.	20% planted within 1 linear mile, ½ mile preferred
TBW and CBW only: Cotton Pest Insect Management Forum	None	10% embedded refuge that is at least 300 ft wide (approx. 80-100 rows); For small or irregularly shaped fields, neighboring fields farmed by the same grower can be grouped into blocks to represent a larger field unit, provided the block exists within one mile squared of the Bollgard cotton and is at least 300 ft. wide. The refuge may be treated as long as the whole field(s) – Bt and non-Bt are treated.	30% planted within 1 square mile area of the Bt cotton (at no point should a Bt cotton field be >1 linear mile from a non-Bt cotton refuge field)
TBW, CBW, and PBW: Gould and Tabashnik (1998)	None	16.7% refuge (eight rows non-Bt cotton for every 48 rows of Bt cotton) – the non-Bt cotton would be planted in at least sets of two or more adjacent rows. The refuge may be treated as long as the whole field(s) – Bt and non-Bt are treated.	50% within 1 square mile area of the Bt cotton for TBW and CBW or immediately adjacent for PBW

Refuge Scenarios	**External Unsprayed (Structured)**	**Embedded**	**External Sprayed**
PBW only: Arizona Bt Cotton Working Group	None	10% embedded refuge in which at least one row of non-Bt cotton must be planted within every six to ten rows of Bt cotton. The refuge may be treated as long as the whole field(s) – Bt and non-Bt are treated.	20% within each square mile of land (one section), non-Bt cotton should be no more than one mile from the leading edge of each Bt cotton field
PBW eradication/ supersession in California: CA Pest Control Board	0% non-Bt cotton:100% Bt Cotton - San Joaquin Valley; include Imperial and Palo Verde	None	None

Gould's and Caprio's Models for TBW and CBW Resistance Management

Gould's Model for TBW and CBW Resistance Management

Dr. Fred Gould, entomologist, North Carolina State University (personal communication to S. Matten, 2000) modeled the performance of several refuge scenarios (see Table below). The model assumes diploid genetics, random mating, three generations per year, an initial resistance allele frequency of 0.001, does not include density dependence, and is deterministic. Gould varied the degree of mortality of susceptible larvae to account for crops with differing compatibility with the high dose concept. He also varied the degree of recessiveness of the resistance alleles. All scenarios were for external unsprayed refuge options.

Gould's Model for TBW and CBW Resistance Management

Fitness of Bt plants RR = (homozygous resistant fitness) ; Rr = (heterozygote fitness); rr= (homozygous susceptible fitness)	**Years to Resistance Allele Frequency Reaching 0.50 for Varied Refuge Sizes (Unsprayed)**			
	4% refuge	**5% refuge**	**10% refuge**	**20% refuge**
Case 1: Extremely high efficacy against susceptible insects RR =1.0; Rr =0.01; rr =0.0001	5.3	6.3	11.0	22.7

Fitness of Bt plants RR = (homozygous resistant fitness) ; Rr = (heterozygote fitness); rr= (homozygous susceptible fitness)	**Years to Resistance Allele Frequency Reaching 0.50 for Varied Refuge Sizes (Unsprayed)**			
	4% refuge	**5% refuge**	**10% refuge**	**20% refuge**
Case 2: Very high efficacy against susceptible insects RR=1.0; Rr=0.01; rr=0.001	5.7	6.7	11.7	24
Case 3 [Case for TBW]: Extremely high efficacy against susceptible insects RR=1.0; Rr=0.001; rr=0.0001	12	14.7	29	62.3
Case 4 [Case for TBW]: Very high efficacy against susceptible insects RR=1.0; Rr=0.002; rr=0.001	12	14.7	28.3	61
Case 5: Moderate/high efficacy against susceptible insects RR=1.0; Rr=0.02; rr=0.01	6	7	12	23.3
Case 6 [Case appropriate for CBW]: Moderate efficacy against susceptible insects RR=1.0; Rr=0.2; rr=0.1	4	4.3	5.3	7.7

Caprio's Model for CBW Resistance Management

Mike Caprio, entomologist, Mississippi State University (personal communication to S. Matten, 2000b) modeled the effect of different refuge scenarios (see Table below) on CBW resistance. Caprio's model assumes that no corn was in the area, so the results are based on CBW being exposed to cotton through four generations/year. Most areas will have a substantial refuge in corn during the first two generations, so this model might represent a worst case (depending on whether or not Bt corn is growing in the area), but not an unlikely one when considering the entire cotton belt. In the model, he assumes 5% survivorship of susceptibles, 2 X 10^{-3} initial gene frequency, and that resistance is a partially recessive trait (h = 0.1). Overwintering survival was estimated to be 25%. Dispersal associated with overwintering and the first spring generation (from non-crop hosts to cotton) was assumed to be 90%. This estimate was probably low, but was used to overcome scale limitations associated with complex simulations. The daily dispersal rate for the first two generations on crop hosts was assumed to be 80%/day. It is assumed that cotton is not a very good host during this time and CBW moves from field to field. Refuges are assumed to be in the same location each year. However, Caprio notes that this shouldn't be a problem given the high overwintering dispersal and high dispersal during the first two generations. Wild hosts are not simulated. For the last two generations, dispersal is set at 25%/day (i.e., 25% of adults leave a patch per day - -a field may consist of many patches, a patch is 10 acres). Caprio calculated that about 46% of the eggs from females emerging in the refuges are laid in the refuge. With dispersal set to 50% per day, 21% of eggs from females emerging in the refuges are laid in the refuge. This is about what Caprio estimated for refuges that are approximately 300 feet wide (67% dispersal parameter). Larval movement is ignored in this model. The number given by the model is years until 50% of the fields have resistance allele frequencies above 50%.

Caprio's Model for *H. zea* (CBW) Resistance Management

Refuge Option	Years to Resistance
Untreated (more like a seed mix or single row)	
4%	3.46 years (+ 2 extinctions)
16%	5.3 years (+ 2 extinctions)
32%	9.5 years
Sprayed external refuges (economic threshold at 4% with 90% efficacy of the larval population)	
0%	2.2
10%	7.25
20%	10.5
30%	14.5
External untreated, structured refuges (refuge 150 ft wide, within ½ mile) New refuge choice for 2001.	Model has not be used to generate this number. However, adding for the 2001 season the structure of at least 150 ft wide refuge within ½ mile should increase estimated years to resistance.

Refuge Option	Years to Resistance
Embedded untreated refuges (50% Dispersal)	
1.25%	8.6
2.5%	10.3
5.0%	19.2
10.0%	24.8
Embedded untreated refuges (67% Dispersal)	
1.25%	7.0
2.5%	8.0
5.0%	12.0
10.0%	22.4

Bt Corn

Questions 8 through 11

A great deal of pest biology, behavior, and ecology information has been gathered for ECB and to a lesser extent for CEW, SWCB, and other stalk-boring pests. Different pest biology can effect the factors to consider for insect resistance management. Proximity, high dose or not, and structure (size and width) of the refugia are important factors.

Hunt al.'s mark-recapture studies (1998-1999) with ECB adult's indicate that more adults were captured within 1500 feet of the field and females were observed to mate within 10 feet of Bt corn fields. These data suggest that a non-Bt corn refuge used to manage European corn borer resistance should be placed relatively close to the field, e.g., within 1/4 to ½ mile.

CEW movement and behavior may be particularly important because of its impact on both Bt corn and Bt cotton systems.

The limited available movement and mating data for SWCB suggests that a non-Bt corn refuge should be planted relatively close to the Bt corn fields, e.g., within 1/4 to ½ mile.

The scientific literature indicates that an in-field refuge may offer the best opportunity for susceptible insects produced in the refuge to randomly mate with rare resistant insects produced in Bt fields. Based on ECB and CEW larval movement information, both insects are capable of moving across rows, but across-row movement is limited.

Models developed by Onstad and Gould (1998), Gould and Onstad (1998), Onstad and Guse (2000), and Hurley et al. (1997) predict that a 20% refuge would delay resistance >>20 years assuming a high dose (for ECB) products and random-mating.

CEW is a particular concern because of its movement from corn to cotton (and other crops) and the consequent exposure of four (or more generations) of CEW to Bt toxins. In addition, current Bt corn products do not produce a high dose for control of CEW. Models by Caprio (see ILSI Report, 1999) predict that in areas in which there is high Bt corn and high levels of Bt cotton that the CBW resistance risk significantly increases. Bt cotton sales data indicate that there have been tremendous increases in Bt cotton acres planted in several Northern Cotton Belt states, especially in Tennessee and North Carolina.

<u>Question 12</u>

Structured refuges for Bt sweet corn were not recommended (nor required) for the following reasons: 1) sweet corn is typically harvested earlier than field corn (18-21) days after silking (before most lepidopteran larvae complete development); and 2) all Bt sweet corn residues were to be destroyed within one month of harvest (a practice that presumably would destroy any live larvae left in corn stalks).

<u>Question 13</u>

<u>*Bt* Cotton</u>

Four years of resistance monitoring information for TBW, CBW, and PBW have indicated no significant changes in susceptibility to the Cry1Ac protein.

TBW and CBW resistance monitoring studies have indicated that there is a statistically-significant increase of CBW tolerance to Cry1Ac (about 10-fold) observed from 1996-1998 in South Alabama, Florida Panhandle, South Carolina, and Georgia, but no shifts in TBW tolerance (Sumerford et al.1999).   Existing monitoring techniques have involved a combination of a diagnostic dose in combination with a growth inhibition assay.   Variable sampling and sample size may have been inadequate in previous years, 1996-1999. To remedy these two areas of concern, Sumerford and Hardee, USDA/ARS/SIMRU (2000a) indicate that they will conduct a more extensive resistance monitoring program for the year 2000. This program will have a uniform collection protocol, increased sample size per location, and use, in part, a more sensitive monitoring technique, F_2 screen.   Implementation of the proposed 2000 monitoring program should improve interpretation, accuracy, and precision of monitoring results.

In the case of PBW, 100 to 400-fold resistance has been selected in the laboratory from more tolerant field populations. These resistant colonies can survive and reproduce on Bt cotton grown in the greenhouse. Further studies on these resistant colonies have shown that resistance was inherited in a recessive fashion, but that there was asynchronous development. Asynchronous development may negatively impact resistance management by impeding random mating, but further study is required to confirm or deny this. Initial resistance allele frequency estimates for PBW were incorrect and, based on data collected in 1997, the resistance allele frequency was significantly higher than the 0.001 estimate in 1995. However, there has been no statistically significant change in PBW susceptibility to the Cry1Ac toxin since Bt cotton was commercialized.

Bt Corn

ECB and CEW resistance monitoring programs have shown no increase in susceptibility to Cry1Ab, Cry1Ac, or Cry9C toxins in commercial field corn hybrids. Limited resistance monitoring information exists for SWCB, but there is no indication of field resistance. Existing monitoring techniques have involved either a discriminating and/or a diagnostic dose bioassay to measure changes in susceptibility.

The Agricultural Biotechnology Stewardship Technical Committee (ABSTC) proposed a tiered approach to resistance monitoring for ECB, CEW, and SWCB. The ABSTC plan focuses resistance monitoring in four major regions where Bt corn market penetration is highest as well as areas with the highest insecticide use. The plan includes the identification of counties growing more than 50,000 acres of field corn (Bt and non-Bt) to focus monitoring efforts. ABSTC's proposed plan is designed to detect resistance when it reaches 1 - 5% (level that allow for detection of resistance before field failures occur). A small shift in allele frequency will not be detected if the ABSTC proposed plan is followed. The full monitoring plan is found in the Agency’s background documents.

Question 14

Bt Cotton

As part of the remedial action plan, the Arizona Bt Cotton Working Group created the Arizona Bt Cotton Rapid Response Team led by the Arizona Cotton Research and Protection Council to investigate field reports of putative resistance and forwards putatively resistant populations to the University of Arizona’s EARML laboratory for testing susceptibility to Cry1Ac. The Rapid Response Team has documented no “in-field” resistance events. The basic components of the remedial action plan are summarized below:

- A resistance event becomes verified if: i) a sample of 2000 cotton bolls yields more than 3% large pink bollworm larvae, pupae, or exit holes, ii) standardized laboratory bioassays (Patin *et al.*, 1999) demonstrate that resistance has a genetic basis, and iii) ELISA tests for the Bt endotoxin provide a positive response for 25 bolls from plants where the PBW survive.

- Delineation of a “Bt resistance remedial action zone” based on sampling of PBW in the area where resistance was found. The remedial action zone should include all sections of land falling within six miles of the perimeter of the section (s) of land in which verified/reportable resistance occurred.
- Use of multiple tactics to suppress the resistant population within the remedial action zone, including: timely crop termination (avoidance of a top-crop) and early cultivation, conventional chemicals, sterile moths, parasitic nematodes.
- Revision of the resistance management plan for PBW for the next year.

- Planting of only non-Bt cotton in the remedial action zone following a verified/reportable resistance event until bioassays demonstrate that the frequency of resistant individuals has declined to acceptable levels.

Sumerford and Hardee (2000) have developed a plan to investigate “problem fields,” where growers experience unusual TBW and/or CBW damage beginning with the 2000 season. Their plan will test progeny from problem fields, use a sublethal diagnostic concentration, and dose-response assay to see if the isolated population falls outside the normal susceptibility parameters determined by baseline data.

Bt Corn

For Bt field corn products, EPA mandated (year 2000) “suspected” resistance to mean, in the case of reported product failure, that the Bt corn in question has been confirmed to Bt corn and that the seed used expressed the expected level of Bt toxin in the expected tissues, and that it has been ruled out that a species not susceptible to the toxin could be responsible for the damage, that no climatic or culture reasons could be responsible for the damage, and that other reasonable causes for the observed product failure have been rule out. EPA has also mandated immediate sales suspension in areas fitting this definition. The detailed remedial action plan is described in the Agency background documents.

Insect Resistance Management Questions

1. What improvements, if any, should be made to the 1998 SAP definition of high dose and its verification?

<u>*Bt* Cotton</u>

2. What impact does differential expression in different *Bt* cotton cultivars have on resistance management for TBW, CBW, and PBW? What data can be collected to investigate the impact of differential expression in different Bt cotton cultivars on refuge strategies?

3. How does CBW north to south movement (and potential gene flow) affect refuge design and deployment for *Bt* cotton? *Bt* corn?

4. EPA believes models are an important tool in its weight of evidence approach to determine which IRM strategy will be most effective in reducing the risk of resistance development. How should the *Bt* cotton insect resistance management models (Gould, Caprio, Peck, Livingston) be used to evaluate the effectiveness (i.e. years to resistance) of potential refuge options? How can these models be verified? How can these models (or others) be improved to more accurately predict when (or if) resistance is likely to occur?

5. Compare and contrast the technical effectiveness (including refuge proximity and structure), grower feasibility, and likelihood of adoption for each refuge option: 95:5 or 90:10 embedded refuge, 95:5 or 90:10 external unsprayed refuge, and 70:30 or 80:20 external sprayed for each of the three primary target pests: TBW, CBW, and PBW? What if any additional refuge strategies that should be considered, e.g. 20% seed mix for PBW?

6. What is the minimum size and structure of a refuge needed to mitigate TBW and CBW resistance if there are multiple small fields (<25A each) grouped to represent an "embedded area" refuge?

7. What is the effect on the production of susceptible lepidopteran insects in the "unsprayed" refuge from the use of a ½ pound rate of acephate and methyl parathion for control of stink bugs or plant bugs? Does the use of pyrethroid oversprays (on the Bt fields) effectively provide a "high dose" for control of CBW? What is the impact of these oversprays on control of CBW in Bt cotton fields? How can management of the refuge be improved with use of appropriate economic thresholds to minimize insecticide treatment, good agronomic practices, economic incentives, and other incentives?

<u>*Bt* Corn</u>

8. How should the following resistance management models be used to evaluate the effectiveness of these refuge options (e.g., years to resistance): Gould and Onstad, Onstad

and Gould, Onstad and Guse, Hurley et al.? How can the models be verified? How can these models (or others) be improved to more accurately predict when (or if) resistance is likely to occur? How does the lack of a high dose for control of CEW affect the predictions of the models? Should CEW and SWCB be included in the models? Why or why not?

9. What is the optimal deployment of a 20% refuge to mitigate ECB resistance: 1) in-field, 2) external unsprayed, and 3) external sprayed (i.e., blocks near or adjacent to fields, perimeter strips around fields, blocks or strips within fields)? How will deployment change for areas coinfested with SWCB? CEW? What is the optimal deployment for an in-field refuge, e.g. number of rows (>2 rows v. >6 rows), in the context of what is known about ECB, CEW, and SWCB larval movement data? What deployment method(s) works best for growers on large acreage? Small acreage?

10. Given differences in biology of the target insect pests (ECB, CEW, SWCB, CSB), can pest specific regional plans be defined, especially where there two or more pests? If so, how?

11. What refuge strategies (or other insect control strategies) should be used to best manage insect resistance in areas with frequent insecticide treatment?

<u>*Bt* Sweet Corn</u>

12. What are the strengths and weaknesses of the Agency's analysis of the resistance management plan for *Bt* sweet corn? Is crop destruction of residues necessary and how should it be accomplished? What crop destruct techniques (e.g., rotary mowing, discing, plowdown) are the most effective? When should crop destruction occur, immediately after harvest, or is within 30 days adequate?

<u>*Bt* Corn and *Bt* Cotton</u>

13. What if any improvements are needed to the *Bt* corn and *Bt* cotton monitoring plans (e.g., number of regions, sampling strategy, consistency of sampling, number of populations sampled and bioassayed, monitoring techniques)? What is the sensitivity of the discriminating or diagnostic dose assays currently in use and what is their utility? What is the relevance of the CBW "tolerance" described by Sumerford et. al. and how should it be examined?"

14. What improvements are needed to the remedial action plans for *Bt* corn and *Bt* cotton? What measures should be employed if resistance is determined to exist? Taking into consideration the need to work with farmers who will be affected (both with resistance and without resistance), how quickly can remedial action measures be implemented? How should the affected area be defined? What level of susceptibility or reduction in resistance

allele frequency would one need to achieve before *Bt* corn and/or *Bt* cotton products could return to the market and resistance would be considered mitigated? What other methods might be used to measure the success of a remedial action strategy?

15. Are grower surveys an effective measurement tool of grower adoption of IRM plans? What other measurement tools are available to measure grower adoption (e.g., Global Positioning Satellite)? What compliance mechanisms (e.g., grower contracts, sales incentives, insurance) does the SAP believe will maximize compliance?

Gene flow/Outcossing Questions

1. Does quantifying risk (*e.g*., hybridization rates, gene introgression) provide adequate means to assess potential environmental impact and determine approval of a plant-pesticide which has wild or feral relatives in the U.S.? If yes, what further risk assessment is warranted to evaluate the risk of outcrossing?

2. Are isolation distances as proposed for certified or registered seed considered as sufficient to mitigate gene flow between Bt-crops and wild or feral populations of sexually compatible species? If not, what distances or measures should be imposed to mitigate outcrossing?

3. Does the panel agree that the gene flow and outcrossing assessment contained in the background document are adequate for the currently registered Bt crops? If not, what additional data or issues should be considered to assess gene flow and outcrossing risks from Bt-expressing plant products?

Background for Bt Soil/Fate Questions

Background for Question 1. EPA has evaluated soil invertebrate toxicity studies (Collembola and earthworms) submitted for registration, and has reviewed published feeding studies on Collembola and an orbatid mite. In addition, toxicity testing of Cry proteins in a number of above ground invertebrates, and continuing field scouting of non-target invertebrates, have revealed little or no apparent adverse impact. The Agency has accepted this testing as adequate for hazard assessment; however, soil dwelling, non-pest Coleoptera have not been tested for *Bt* Cry3A in potato tubers.

Background for Question 2. Some recent studies have shown persistence of *Bt* proteins under certain conditions to be much longer than have other studies. Microcosm studies often show initial rapid degradation of most of the added Cry protein, with longer persistence of low concentrations of residual Cry protein.

Background for Questions 3 and 4. A recent report by Saxena et al. in the journal "Nature" concluded that Cry1Ab is exuded from the roots of Bt11 corn (exudation is understood here to represent active deposition by roots into the growth medium, rather than small amounts incidentally lost from roots). EPA concluded that exudation was not clearly demonstrated, although the possibility of exudation also cannot be eliminated based on the Saxena et al. paper. Our understanding of the biochemical requirements for active protein secretion by plant roots suggests that exudation is not likely to occur in Bt crops. EPA believes that available data on degradation of high levels of Cry protein in soil, which does not directly consider the possibility of Cry protein exudation, as well as reviewed studies on non-target soil organisms, are adequate for soil risk assessment.

Background for Question 5. Submitted toxicity tests for soil organisms have been high dose, subchronic tests (and one study of soil invertebrates in the literature involved continuous feeding, with examination of effects on fecundity and mortality). These tests have been considered adequate, since no adverse effects have occurred during these tests to trigger the need for longer term testing. In addition, the basis of exposure of soil organisms to Cry proteins has previously been considered to be primarily from a single incorporation of crop debris at the end of the growing season. However, exposure to soil organisms is likely to be continuous throughout the growing season in Bt crops that express Cry proteins in their roots, regardless of whether exudation occurs. Roots, as well as incorporated plant material, also act as a source of exposure to transgenic DNA.

Bt Soil/Fate Questions

1. Considering that EPA now requires toxicity studies for Collembola and earthworms, what are the appropriate indicator species that should be tested to assess risks of *Bt* Cry proteins on soil invertebrates? In particular, which if any, soil dwelling non-pest Coleoptera could be tested in laboratory conditions that would provide valuable information for assessing risks from Cry3A?

2. The Panel is requested to address whether the studies determining rates of degradation of Cry proteins in soil have been of sufficient duration, and were performed under adequate conditions (typically soil microcosms). Comment on whether available experimental results and EPA's evaluation of this data adequately address the question of persistence of Cry proteins in Bt crop soil.

3. Please comment on what would be appropriate methods to examine secretion of Cry proteins from roots and the merits of such tests for risk assessment (e.g., tests could include examining the protein sequence of Cry proteins for putative endoplasmic reticulum signal peptides or actual experiments to test for secretion). If the Panel believes that testing for secretion is needed, should current Bt crops be tested?

4. Comment on the available data concerning the possibility that Cry protein could accumulate in crop soil and what, if any, additional testing of field soil is needed to adequately address this question for the purpose of hazard assessment.

5. Please provide comment on whether the environmental fate data and horizontal gene transfer assessment is an adequate evaluation of the fate of *Bt* proteins and assessment of horizontal gene transfer? Also, are there additional data, such as that listed by EPA in the preliminary assessment, that should be obtained for the current *Bt* plant-pesticides?

Background for Non-target Organisms Effects Assessment Questions

Question1. The weight of evidence from the reviewed data indicate that there is no hazard to wildlife from the continued registration of *Bt* crops. The Agency evaluated studies of potential effects on a wide variety of non-target organisms that might be exposed to the *Bt* protein expressed in potato, corn, and cotton.- i.e. wild mammals, birds, invertebrate, and aquatic species. EPA concluded that these species were not harmed, nor that *Bt* crops would threaten the long-term survival of a substantial number of individuals in the populations of these species.

Question 2. Preliminary modeling on the overlap of corn pollen shed timing with monarch breeding indicates that, for most of the corn belt except for the northern range and higher elevations in the Northeast, monarch larvae are not present during pollen shed. Where there is overlap, considerations of milkweed distribution in the corn fields, on and off-field corn pollen deposition on milkweeds, and oviposition and larval feeding patterns indicate that the probability for adverse effects on non-target lepidopteran larvae from *Bt* corn is very low. Therefore exposure of monarch larvae to MON810, Bt11 and CBH351 *Bt* corn pollen at levels above the NOEC may not occur in the field. The Agency concludes that continued cultivation of *Bt* corn is not sufficient to cause undue concern of harmful widespread effects to monarch butterflies at this time.

Question 3. Toxicity data show that the only endangered species of concern are in the Lepidoptera and Coleoptera group. The majority of endangered species in these Orders have very restricted habitat range and do not feed on, or approach the *Bt* crop planting areas close enough (within 2 meters) to be exposed to toxic levels of *Bt* pollen. The major concern regarding potential range overlap with *Bt* crop production is restricted to the Karner blue butterfly in the northern corn maturity zones where Karner blue occurs. However, the sole Karner blue host plant, the wild lupine, also does not occur in or in close proximity to corn fields. Even if wild lupine plants were to occur in close proximity to corn, it is highly unlikely that any corn pollen would be present on them at the time Karner blue larvae are actively feeding because there does not appear to be an overlap in corn pollen shed and Karner blue oviposition.

Non-target Organism Effects Assessment Questions

1. The Panel is requested to provide comments on the Agency's weight of evidence assessment and its conclusion that *Bt* crops would not threaten the long-term survival of a substantial number of individuals in the populations of wild mammals, birds, invertebrates, and aquatic species.

2. The Panel is requested to comment on the Agency's analysis of the currently available data on the potential impacts of MON810, Bt 11, and CBH351 on monarch butterflies.

3. The Panel is requested to comment on the Agency's assessment that Karner blue butterflies are not at risk from the current *Bt* plant-pesticides and to provide EPA advice on any further considerations that should be made for this or other endangered species.

4. Please comment on additional studies which might be needed to strengthen the database identified at the end of the environmental assessment including the future on-going research on non-target Lepidoptera and other non-target invertebrate species.

Background for Benefits and Economic Analysis Questions

Question 1. Sensitivity analysis has shown that estimation of variance is much more important than distribution assumptions (uniform vs normal).

Question 2. Estimates of use reduction have relied upon publicly available data from USDA/National Agricultural Statistical Service (NASS). Since target pest data are not contained in the NASS data, the approach compared use reduction for all insecticides and those that controlled the lepidopteran pests controlled by Bt. The analysis also compared use reduction between adopter and non-adopter states. Data were not used for states that were surveyed for some but not all years.

Question 3. Characterization of environmental and health benefits is typically difficult quantify. The benefits section relied upon outcome oriented incidence data, comparing the associated use reduction with those pesticides that account for the highest incidence reports.

Benefits and Economic Analysis Questions

1 Discuss whether there are improvements in the model or methods for estimating benefits and costs? What methods would you suggest to improve estimation of the mean and variance of grower demand (willingness-to-pay)? Would dividing the analysis into more homogeneous geographical units (i.e. infestation, weather, geography, acres planted) be appropriate? Why or why not?

2. Is there a better methodology to incorporate all the NASS data than EPA used it its assessment? Discuss whether the data support more rigorous statistical tests on significant differences.

3. Please provide comments on other approaches which might better characterize and/or quantify environmental and health benefits?

4. Is the benefits assessment contained in the background document an adequate assessment of the benefits from *Bt* plant-pesticides? If not, what additional data are necessary to assess the benefits from *Bt* plant-pesticides?

Product Characterization and Human Health Questions

1. Please provide advice on whether there is a threshold amount of protein below which concern for risk from exposure/consumption of proteins expressed in plants will be eliminated/reduced? If so, how should this threshold be determined?

2. Please provide comment on the quality and thoroughness of the product characterization review. What additional data, if any, should be evaluated in order to adequately characterize the Bt-expressing plant-pesticide products?

3. Please provide comment on whether the human health data is an adequate evaluation of the risk from the Bt proteins. What, if any, additional data is necessary to assess the risk from the Bt-expressing plant-pesticide products?