SAP Report No. 2000-07
March 12, 2001

REPORT

FIFRA Scientific Advisory Panel Meeting, October 18-20, 2000, held at the Marriott Crystal City Hotel

Sets of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Bt Plant-Pesticides Risk and Benefit Assessments

NOTICE

This report has been written as part of the activities of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). This report has not been reviewed for approval by the United States Environmental Protection Agency (Agency) and, hence, the contents of this report do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

The FIFRA SAP was established under the provisions of FIFRA, as amended by the Food Quality Protection Act (FQPA) of 1996, to provide advice, information, and recommendations to the Agency Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the EPA, Office of Pesticide Programs (OPP) and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. Food Quality Protection Act Science Review Board members serve the FIFRA SAP on an ad-hoc basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP reports and activities can be obtained from its website at http://www.epa.gov/scipoly/sap/ or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Larry Dorsey, SAP Executive Secretary, via e-mail at dorsey.larry@.epa.gov.

TABLE OF CONTENTS

SAP Report No. 2000-07a, March 12, 2001

REPORT:
FIFRA Scientific Advisory Panel Meeting,
October 18-20, 2000, held at the Marriott Crystal City Hotel,
Arlington, Virginia

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Bt Plant-Pesticides Risk and Benefit Assessments: Insect Resistance Management

Mr. Paul Lewis
Designated Federal Official
FIFRA Scientific Advisory Panel
Date:_____

Christopher Portier, Ph.D.
FIFRA SAP Session Chair
FIFRA Scientific Advisory Panel
Date:_____

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
October 18-20, 2000

Bt Plant-Pesticides Risk and Benefit Assessments: Insect Resistance Management

PARTICIPANTS

FIFRA SAP Session Chair
Christopher Portier, Ph.D., National Institute of Environmental Health Sciences, Research Triangle Park, NC

FIFRA Scientific Advisory Panel
Stephen Roberts, Ph.D., University of Florida, Gainesville, FL

FQPA Science Review Board Members
David Andow, Ph.D., Department of Entomology, University of Minnesota, St. Paul, MN
Ralph Bagwell, Ph.D., Louisiana Agricultural Center, Winnsboro, LA
Michael Caprio, Ph.D., Department of Entomology, Mississippi State University, Mississippi State, MS
Tim Dennehy, Ph.D., Department of Entomology, University of Arizona, Tucson, AZ
Brian Federici, Ph.D., University of California, Riverside, Department of Entomology and Graduate Programs in Genetics and Microbiology, Riverside, CA
Fred Gould, Ph.D., North Carolina State University, Department of Entomology, Raleigh, NC
Dick Hardee, Ph.D., USDA, ARS, Southern Insect Management Research Unit Stoneville, MS
Richard Hellmich, Ph.D., USDA, ARS Corn Insects and Crop Genetics Research Unit, Ames, Iowa
Randy Higgins, Ph.D., Kansas State University, Department of Entomology, Manhattan, KS
Terrance M. Hurley, Ph.D., Department of Applied Economics, University of Minnesota St. Paul, MN
Mark Sears, Ph.D., Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada
Tony Shelton, Ph.D., Agricultural Experiment Station, Cornell University, Ithaca, NY
Blair Siegfried, Ph.D., Department of Entomology, University of Nebraska, Lincoln, NE

Designated Federal Official
Mr. Paul Lewis, FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, Environmental Protection Agency, Washington, DC

PUBLIC COMMENTERS

Oral statements were made by:
Mr. Dan Moellenbeck, on behalf of ABS Technical Committee
Mr. David Guyer, on behalf of Novartis Seeds
Mr. Hollis Isbell, on behalf of the American Cotton Producers
Jane Rissler, Ph.D., on behalf of the Union of Concerned Scientists
Gary Herzog, Ph.D., on behalf of the University of Georgia
Mr. Larry Antilla, on behalf of the Arizona Cotton Growers Association
Mr. Jesse Young, on behalf of Young and Young
Michael Phillips, Ph.D. on behalf of the Biotechnology Industry Organization
Harold Coble, Ph.D. on behalf of the Council for Agricultural Science and Technology
Galen Dively, Ph.D. on behalf of the University of Maryland
Mr. Jere White, on behalf of the Kansas Corn Growers Association and the Kansas Corn Commission
Mr. Paul Bertels, on behalf of the National Corn Growers Association
Mr. Graham Head, on behalf on Monsanto
David Hawthorne, Ph.D. on behalf of the University of Maryland
Doreen Stabinsky, Ph.D. on behalf of Greenpeace
Ms. Beth Roberts, on behalf of the Center for Food Safety
Michael Ginevan, Ph.D. on behalf of Ginevan and Associates
Rebecca Goldburg, Ph.D. on behalf of Environmental Defense

Written statements were received from:
Agricultural Biotechnology Stewardship Technical Committee
Auburn University
Aventis CropScience
Council for Agricultural Science and Technology
Dow Agrosciences, Inc.
Monsanto
National Cotton Council
Novartis Seeds, Inc.
Pioneer Hi-Bred Inc.
Union of Concerned Scientists

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency pertaining to Bt Plant-Pesticides Risk and Benefits Assessment: Insect Resistance Management. Advance notice of the meeting was published in the *Federal Register* on September 6, 2000. The review was conducted in an open Panel meeting held in Arlington,

Virginia, on October 18, 2000. The meeting was chaired by Christopher Portier, Ph.D. Mr. Paul Lewis served as the Designated Federal Official. Sharlene Matten, Ph.D. (EPA, Office of Pesticide Programs), Ms. Robyn Rose (EPA, Office of Pesticide Programs) and Mr. Alan Reynolds (EPA, Office of Pesticide Programs) discussed the Agency's insect resistance management proposal.

In preparing this report, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This report addresses the information provided and presented within the structure of the charge by the Agency.

CHARGE

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

Bt Cotton

2. What impact does differential expression in different *Bt* cotton cultivars have on resistance management for tobacco budworm (TBW), cotton bollworm (CBW), and pink boll worm (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 insect resistance management (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?

Bt Corn

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 European Corn Borer (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, corn stalk borer [(CSB]), can pest specific regional plans be defined, especially where there are 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?

Bt Sweet Corn

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?

Bt Corn and *Bt* Cotton

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 System)? What compliance mechanisms (e.g., grower contracts, sales incentives, insurance) does the SAP believe will maximize compliance?

DETAILED RESPONSE TO THE CHARGE

The specific issues to be addressed by the Panel are keyed to the Agency's background document "Preliminary Risk and Benefit Assessment for Bt Plant-Pesticides", dated September 20, 2000, and are presented as follows:

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

The Panel generally agreed with the 1998 EPA-SAP which concluded that a refuge/high dose strategy must be used within the current understanding of the technology. Some members of the Panel believed that the limit for high dose should be increased, but the majority of the Panel concluded that there was insufficient need to warrant a change. One recommendation was that the Agency should clarify that the 25X definition of high dose is a provisional one, and that their determination of high-dose status for any Bt crop may be changed if it is determined that the inheritance of resistance in insects from a Bt crop in the field is such that the 25X definition is inappropriate.

The Panel specified that a high dose plant would be one that expressed the toxin at 25 times the dose required to kill 99% (LC99) of a susceptible insect pest population. The 1998 SAP provided five approaches for judging whether or not a specific cultivar met this standard. The logic behind this approach is explained in detail in a number of scientific papers (e.g. Roush 1997,

Andow and Hutchison 1998, Gould and Tabashnik 1998) as well in the 1998 SAP report. Briefly, these studies concluded that for the refuge/high dose approach to be effective, Bt cultivars must produce a toxin concentration that is high enough to kill most insects which are heterozyogous for resistance. The 1998 SAP assumed that a toxin concentration that was 25 times the concentration that would kill susceptible insects would be sufficient to kill heterozygotes. Following the 1998 SAP meeting, Caprio (Caprio et al., 2000) examined all of the empirical data available on survival of heterozygotes compared to susceptible insects. Based on the statistical properties of dose/mortality bioassays, LC50s, rather than LC99, were used in making this determination. Because the slopes of the dose/mortality curves for susceptible and heterozygote insects were generally similar, this approach was considered valid. Of the 17 cases examined, Caprio found that four cases (23.5%) exceeded the 25- fold rule. If the high dose definition used a 50-fold limit, only one case would exceed this limit. Caprio, therefore, suggested that the high dose requirements be increased.

A number of public commenters challenged the need for raising the high dose limits. Some of the concerns expressed included: 1) the heterozygotes tested to date do not survive on current high dose cultivars, 2) in some of the cases used by Caprio, inheritance of resistance was dominant and, 3)use of the LC50 values is not appropriate and; 4) increasing the dose beyond 25-fold might exacerbate problems associated with non-target exposure.

The SAP discussed these and other issues related to the high dose definition. In general, the Panel indicated that no single number could be rigorously defended as the cut off for a high dose for any key pest species at the present time, because the resistance properties of every resistance allele in the field cannot be predicted. Any definition of high dose must therefore be imprecise.

Some members of the Panel explained that the mortality of heterozygotes on current cultivars provides evidence, in addition to the 25X data, that these cultivars meet the high dose requirements. However, mortality of heterozygotes could result from less than 25X resistance or because the cultivar has more than the 25X dose of Bt toxin. Information on the Colorado potato beetle (CPB) and the European corn borer (ECB) indicates that some of the potato and corn cultivars tested caused mortality of larvae with at least 157 and 60+ fold resistance, respectively. This information indicates that these current cultivars have Bt titers that significantly exceed the 25X criteria. Panel members pointed out that there is no real concern over these current cultivars, but with other potential cultivars which may barely meet the 25X criteria. Of such cultivars, insect strains with 157 or 60+ fold resistance would be expected to survive.

Some Panel members also addressed the issue of whether the 25X limit was valid only if resistance was inherited as a recessive trait. The conclusion was that even if a resistance trait was dominant, survival of a substantial portion of heterozygous individuals on a cultivar would be problematic. Such survival would lead to rapid increase in frequency of the resistance alleles, and both heterozygotes and homozygote resistant individuals would survive on the cultivar.

It was pointed out that determination of whether one has a 25X or 50X dose is complicated by the natural variation of the targeted insect pest. For example, Stone and Sims (1993) found up to 8X and 16X variation to purified Cry1Ac endotoxin, for TBW and CBW, respectively, in samples originally collected from geographically distinct field populations. If the population of insects chosen for testing the high dose compliance was on the low or high end of those available, the standard for high dose would differ considerably. Other Panel members commented that it was therefore helpful that the 1998 SAP recommendation mentioned the need for use of more than one type of test to determine if a cultivar could be considered to produce a high dose.

Concern was expressed that the current 25X definition of high dose was not being enforced and that some cultivars being grown in the US did not even cause 99% mortality of susceptible insects (e.g., CBW on corn and cotton).

***Bt* Cotton**

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?

The Panel concluded that the impact of differential expression of Bt toxin among cultivars on resistance management is dependent upon the level of toxin expression and the species of insect. As long as the expression level of all cultivars exceeds the definition of "high dose" then variation among cultivars should not be a cause of concern. If the expression level of any cultivar is below that of the defined "high dose," then the impact on resistance development could be significant. Although there are few publications on variation among cultivars, the Panel consensus was that for TBW and PBW, all current cotton cultivars in the US probably produced a high dose. For CBW, none of the current cultivars produce a high dose. Variation among cultivars could impact the rate of CBW adaptation but the major problem remains that none of the cultivars produce a high dose.

CBW strains with 100 fold resistance have been developed in Mississippi and North Carolina. The North Carolina strain was shown to have higher survival than susceptible strains on Bt cotton. One strain with resistance was developed after only one generation out of the field (Burd et al., 2000).

It was pointed out that in addition to variation among pure cultivars in Bt expression level, another cultivar-related characteristic that could affect the high dose was the degree of purity of the seed sold. If the seed sold had a significant percentage of non-expressing plants and larvae move among plants, this could negatively impact resistance management.

3. How does CBW north to south movement (and potential gene flow) affect refuge design

and deployment for *Bt* cotton? *Bt* corn?

The Panel discussed the Agency's current requirement of a 20% corn refuge in the North and a 50% refuge in the South which is based on CBW being exposed to both Bt corn and Bt cotton in the South. The Agency uses strict political boundaries in distinguishing North from South. This approach makes the assumption of no return migration in CBW.

The Panel reviewed data relevant to the question of whether CBW migrates both to the North in early summer and to the South in late summer. The northward migration is more clearly documented than the southward migration. The general consensus was that although southward migration was not proven, there was considerable circumstantial evidence of southward migration, and it should therefore be considered in resistance management. There is more evidence supporting southward movement than evidence against southward movement. The need for further scientific investigation of CBW migration was emphasized.

The Panel discussed how resistance management for Bt corn and Bt cotton could be impacted by North-South migration. Such migration would mean that selection pressure from Bt corn in the corn belt, as well as the refuge provided by non-Bt corn in the corn belt, should be considered in resistance management.

Since Bt cotton and Bt corn do not produce a high dose for CBW, plus the probable long-distance, cross continental movement of CBW from the northern corn producing areas to the southeastern corn and cotton producing areas of the United States, the exact placement of refuges is not as critical as it is with insects for which there is an appropriate high dose. This means that the percentage of Bt corn planted in some subregion of the northern corn belt should be more important to resistance management than the percent refuge on each farm. As long as the percent corn planted in such a region is below 50%, then the 20% refuge requirement for each farm in the corn belt may not be a concern. If the overall percentage of Bt corn in a region increases above 50%, then the issue of on farm refuge size may need to be reconsidered.

If further studies demonstrated that migrating moths from specific corn belt states with high percentages of non-Bt corn contributed significantly to the following season's CBW population, then non-Bt contributions of CBW from corn could be considered in determining the cotton refuge size.

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?

The Panel discussed a number of written and oral public comments presented at the SAP

meeting, which pointed out limitations of mathematical simulation models used in determination of resistance management plans. The Panel also reviewed the assertion, made during the public comment period, that models have made false predictions because resistance has not yet been seen. The Panel responded to these and other concerns about the use of these models.

It was the consensus of the Panel that models were an important tool in determining appropriate Bt resistance management plans. The Panel agreed that mathematical models were the only scientifically rigorous way to integrate all of the biological information available, and that without these models, the Agency would have little scientific basis for choosing among alternative resistance management options. However, Panel members felt that there was a need to better define just how to weigh the results of models in decision making, and how to judge the accuracy and applicability of specific models.

It was pointed out that all mathematical models are simplifications of real systems and by definition, are inaccurate. Furthermore, mathematical models can only be as good as the data that are used to parameterize them. This is just as true of the mental models that we use every day in decision making. The difference between mental and mathematical models is that the latter make explicit all the assumptions that determine the result. This means that mathematical models are much more amenable to rigorous scrutiny than mental models. The fact that successful industries typically use mathematical models in making technical and economic decisions was brought up by a Panel member as *de facto* evidence that mathematical models are considered useful.

Some Panel members and public commentators pointed out limitations of some resistance management models that did not consider alternate hosts, population dynamics, or stochasticity. Panel members familiar with modeling pointed out that there has been a progression in modeling from simple to more complex models, and that some of the current models do consider the issues mentioned above.

Because a model can only really be considered to be completely validated when the predictions of the model are shown to correspond to the real world, some Panel members felt that it is impossible to completely validate resistance management models until resistance develops. However, components of resistance management models can be validated prior to the development of resistance. For example, a model can be examined to determine if the population dynamics of the still susceptible population match the observed field population dynamics. A model could also be shown to be false if it predicts resistance, and resistance does not evolve. It was pointed out that the currently available, detailed population genetics models for cotton did not predict that resistance would be detectable in the year 2000, given the amount of Bt cotton planted in the US since 1996.

What biological assumptions are used in a model can sometimes depend on whether the model is being used to look at the best, worst, or commonly expected biological parameters. One example brought up in public comments at the meeting and by the Panel was the issue of fitness costs to resistant individuals. Most models don’t assume such a cost because resistant strains

have been found that do not revert to susceptibility. This does not mean that a fitness deficit will never be observed and that under certain circumstances, a fitness cost may be observed. Future modeling efforts should consider this possibility. Another example is that models of CBW do not consider soybean as a refuge because most information indicates that it isn't a reliable host each season. A best case scenario model would include soybean as a refuge. This points out that if there were better empirical data on soybeans, a more realistic model could be developed that accounted for the true year to year variation in the utility of soybean as a refuge.

The Panel commented that the entire refuge/high dose strategy is based on the assumption that there is no dominant gene that would enable larvae to survive on Bt cultivars. If such a gene exists, refuges would need to be much larger to slow resistance development. In this regard, all models to date are using optimistic criteria.

A number of criticisms of reliance on models pointed to the fact that models don't take into account the feasibility and economic impact of specific resistance management plans. The Panel commented that economic considerations are being added to models. Because adding economics to the models requires quantitative parameters, this work has forced the question of "How to determine the appropriate currency for such economic/biological models?" The currency (or concern) is very different if we are trying to produce optimal benefits for commercial cotton farmers, cotton seed producers, consumers, or organic farmers. If the benefits for conventional cotton farmers is the major concern, a five year time horizon may be appropriate. However, if the desires of the organic farmer are the major concerns, a much longer time span needs to be considered. Modelers need a more specific directive if they are to specify parameters appropriately.

In this same regard, most resistance management models have focused on Bt resistance, although some Bt resistance strategies for cotton can affect the evolution of resistance in TBW, CBW and PBW to other insecticides (Livingston et al., 2000, Caprio, 1998). How should resistance management plans weight the importance of resistance to Bt versus conventional pesticides?

The Panel generally believed that in addition to the peer review of models that is accomplished during the typical publication procedure, there is a need to establish an approach for detailed peer review of the components of each model used by the Agency for establishing resistance management plans.

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?

Almost all Panel members agreed that the 5% external unsprayed refuge could be used if

there was 100% compliance and a monitoring program was instituted that could identify resistance at low allele frequency. One Panel member stated at the outset of the discussion that if a refuge approach is too hard to comply with, we are wasting our time trying to get compliance. Two members of the Panel agreed that even if 100% compliance and monitoring could be accomplished, the 5% unsprayed external refuge should not be an option. The strong concerns of the Panel with the 5% external refuge were that it is of minimal size, and farmers have a history of not complying with its use. The Panel generally believed that there was a real balance needed between feasibility and use of a specific refuge plan.

In terms of sprayed external refuges, there was discussion over whether the distance between Bt and non-Bt fields should be less than 1 mile or less than ½ mile, but there was no resolution to this discussion.

Regarding the comparison of the 95:5 and 90:10 embedded refuge, one Panel member stated that: (a) assuming equal compliance, 90:10 will be more effective as it will likely produce 2 x or more refuge individuals for mating with resistant allele carrying moths. If equal numbers of units are deployed in a region, the 90:10 will have bigger plots and thus be less influenced by source/sink effect (i.e., less recycling of generations within the refuge). However, if deployed in equal size units, the 90:10 will produce roughly 2 x the refuge moth numbers as the 95:5. The 90:10 also has a benefit since more moths emerging from more refuge units will likely come closer to random mating than vice-versa and; (b) computer simulation indicates less difference in resistance delay between the two options with TBW than with bollworm (BW). In this case, it is BW that dictates the refuge size since its genetics and susceptibility make it more prone to develop resistance rapidly. Due to a high dominance and resistance gene frequency in BW, even a 10% embedded refuge may be inadequate.

Another member of the Panel stated that the data clearly show that some type of embedded refuge would delay resistance evolution of the insect so it overcomes Bt-cotton the longest. In addition, such a refuge would be effective for every toxin used in the cotton agroecosystem and not be limited to Bt-cotton. Unfortunately, such an embedded system would require significant effort from companies, growers, consultants and regulators. The alternative to an embedded refuge is a sprayed refuge. The Panel member did not support a sprayed refuge since it advocates simultaneous use of two technologies in the same time frame where one technology would normally be used. A sprayed refuge option utilizes susceptibility to both Bt and conventional insecticides (something not considered in the current recommendations of 20-30% sprayed refuges). Thus, the Bt-acreage at the grower or community level should be limited to 50%.

The situation in the Southwestern US is very different than in other areas of the country because the PBW has very limited larval movement. One Panel member presented the following table summarizing approaches for the Southwest (Table 1).

Table 1: Pink Bollworm Refuge Options in the Southwest

Pink Bollworm - Southwest			
Refuge Option	**Technical Effectiveness**	**Grower Feasibility**	**Adoption Likelihood**
95:5 embedded/unsprayed	Okay, but only if planted as blocks. No single rows	OK	Low. External blocks are less trouble
90:10 embedded/unsprayed	Very good. May be single rows. No Lepidopteran treatments to non-Bt row	Good	Good. But not applicable to seed production situations
95:5 external/unsprayed	Good, if planted within 1 mile	Good	Good
90:10 external/unsprayed	Very good, if planted w/in 1 mile	Very poor	Very poor. Unacceptable yield loss
70:30 external/sprayed	Very good, if planted within 1 mile	Very poor	Very poor. Unacceptable yield losses
80:20 external/sprayed	Good, if planted within 1 mile	OK	OK. Most growers opt to use 5% external refuge. Note subsequent to the meeting, the Panel member determined that most growers now use the 20% external refuge.

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?

The Panel concluded that there was no reason to have a different size refuge (on a percentage acreage basis) for small fields versus large fields. It was clear that for very small fields, the embedded refuge would not be feasible.

One Panel member believed that by permitting producers to share refuges, it is possible to provide a stronger incentive for community-based pest management. Sharing of refuges will require that growers invest more into identifying where refuges are placed in their community. This will increase producer interest in mapping of Bt/non-Bt cotton and will likely help to reduce non-compliance, as refuge placement will need to be mapped on a community level.

It was also noted that although many people directly equate size of refuge with effectiveness, what is really important is the number of susceptible moths produced in a refuge that will mate with insects in the Bt crop. It may be useful to consider the conclusions from the 1998 SAP report that there should be 500 adults produced in the refuge for each resistance allele-carrying adult produced in the Bt crop.

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?

There was clear consensus among Panel members that pyrethroid oversprays would not provide a high dose for control of CBW. The Panel believed that compliance with the Agency's requirements that the refuge cotton be grown in a manner similar to the non-refuge cotton could increase the effectiveness of refuges compared to the current situation where some of the refuge areas are underfertilized, grown on poor soils, or planted late.

Any action that results in fewer susceptible insects emerging from the refuge will accelerate the evolution of resistance. Thus, for effective IRM, any management practice that does not eliminate insects from the refuge will be helpful. Consequently, use of economic thresholds is better than prophylactic insecticide applications to the refuge, and any incentives that encourages a grower to avoid controlling pests on the refuge will be helpful.

One Panel member pointed out that at 0.5 lb (AI)/A, neither methyl parathion nor acephate is expected to provide much mortality in field populations of either CBW or TBW. This is especially true for methyl parathion. The positive benefits may be numerous. First, acephate at 0.5 lbs (AI)/A, may delay CBW/TBW developmental time during the larval stages. Thus, applications of acephate may increase generation time to coincide with any delays in development associated with Bt cotton. Methyl parathion at 0.5 lb(AI)/A will probably have no effect on CBW or TBW larvae generation time. Second, both compounds are considered to be harsh on beneficial insect populations. Thus, application of either insecticide will greatly reduce a major mortality factor associated with both species in the refuge area. For example, many researchers will commonly apply methyl parathion or acephate to field plots where CBW/TBW experiments are to be conducted. These applications will usually result in higher CBW/TBW populations in

the experimental area. The third benefit also has some negative points in association with IRM. Both insecticides reduce numbers of other insect pest populations, such as the plant bug complex and stink bug complex. These two complexes feed on fruit. Reduction in these insect pest populations increases the amount of substrate available for CBW/TBW development. This increase in fruiting forms available will, however, result in earlier maturity of the refuge crop. As such, the positive and negative probably cancel one another out.

***Bt* Corn**

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?

Most of the Panel concluded that this question was similar to question 4. The Panel generally agreed that the predictive power of models will always have limits, but that models are currently the only transparent means available for evaluating alternative IRM plans. The goal must be to improve both the models themselves and the data used to initiate the models.

Beyond that, it was emphasized that the Agency needs to maintain leadership in determining the goals of resistance management and how these goals should be weighted by scientists who are developing resistance management plans. One Panel member indicated that some models use insect resistance to Bt, while others use grower profit as the currency to compare IRM strategies.

One Panel member commented that the Onstad/Gould model had mostly been developed to examine concerns about use of Bt corn cultivars that involved transformation event 176 which will no longer be registered. The Panel agreed that SWCB and CEW should be modeled. It will be extremely difficult to devise an appropriate management approach without these models. It would be best to develop some models that take multiple pests into account. The Panel emphasized that the lack of a high dose for the CEW/CBW generally means that refuge sizes must be much larger.

In terms of improving the models and data, there were a number of specific comments. As computer processing technology improves and more complicated models become more feasible, the Panel recommended adding spatial (patchwork) and stochastic components. Also, several Panel members strongly recommended that sociological components such as grower compliance should be added to the models. Panel members also recognize a communication gap between modelers and field biologists. Efforts should be made to facilitate discussions between these groups so that field and biological realities can be better incorporated into the models.

Our current knowledge of ECB biology and genetics indicates that the general assumptions

of the refuge/high dose strategy may be met, but data are lacking to confirm this. For example, some data are available from F_2 screening indicates that the frequency of recessive resistance alleles are in line with assumptions of the refuge/high dose strategy, but more information is needed on the presence of functionally dominant resistance alleles. Some information on movement of adults, which is useful for determining distances between Bt and non-Bt fields is available, but we must determine if moths move before or after mating to make this information truly useful for parameterizing the models.

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?

The Panel felt that proper refuge deployment requires that mating and movement patterns for primary pests be understood. However, information for most target insect pests, such as ECB is still emerging. The objectives of refuge design and placement should be to: 1) provide a source of susceptible homozygotes which optimizes the probability of mating between these susceptible insects and the few resistant insects that may emerge from Bt corn fields, 2) minimize the movement of larvae between Bt and non-Bt corn plants, and 3) allow flexibility for growers with varied cropping practices and needs.

Regarding objectives 1 and 2, an in-field refuge of blocks or strips of non-Bt corn is likely to maximize random mating. Importantly, a refuge must have similar agronomic traits (e.g., maturity) to the Bt acreage and be managed similarly to maximize synchronous development of ECB in refuge and Bt acres. Strips of refuge should be wide enough (at least >6 rows) to minimize potential movement of RS genotypes from Bt to non-Bt corn. The Panel felt that whole field refuges were a viable option but recommended that these refuges should be located no further than a half mile (within1/4 mile if possible) from the Bt corn field.

Mark-release-recapture experiments have produced more modest dispersal values. Marking insects in the field with rubidium, Legg (1983) found that second generation moths can disperse 3 km. Showers (in press) marked laboratory insects with vital dyes, and recaptured males at pheromone traps at 4 distances (200, 800, 3200 and 6400 m) during the second flight period. About 60% were recaptured at the closest traps (200m) and 6% were caught at the two more distant traps. Hunt (1999) also conducted a mark-release recapture experiment using laboratory insects marked with vital dyes, but recaptured insects at light traps so both male and female moths were recaptured. Nearly all of the recaptured unmated females were within 500 m of the release site during the second flight period.

The Panel stated that there was insufficient data on SWCB to warrant modification of the

current refuge for most of the Midwestern corn belt. This may not be true for areas commonly treated for SWCB, which will be discussed later under question 11. Information on CEW, as discussed under question 3, indicates that under current conditions of market penetration, it is unlikely that refuge recommendations need to be modified to accommodate this insect.

The third objective of refuge design discussed by the Panel was allowing flexibility for growers with varied cropping practices and needs. It was pointed out that the lack of grower compliance could seriously undermine resistance management efforts. The Panel concluded that the provision of flexible but scientifically defensible options to growers could increase compliance by instilling confidence in the process and by increasing the general feasibility of planting refuges.

Growers should have a variety of refuge options (blocks, within-field strips, border rows, etc.) for IRM so that they can choose the option that best fits their equipment and farming practices. However, one option that should be discouraged is the use of seed mixtures that would result in substantial larval movement between Bt and non-Bt plants.

There was disagreement among the Panel members regarding acceptable dimensions of in-field refuge. The Panel generally agreed that wider refuge strips ($\geq$ 6 rows) are better than narrower strips because of larval movement considerations. There was some concern that mandating 6 rows or more for strip size could discriminate against growers with small (< 12 row) planters. For growers using these smaller planters, in-field block refuges may be quite feasible. For example, a grower cannot plant an 80 acre field with a 6 or 8 row planter without having to refill the planter boxes. Thus, it would be possible to plant a non-Bt block within the field (as long as the grower was not required to vacuum out the Bt seed before adding the non-Bt seed). In such cases, the grower should first be encouraged not to use strips. However, if no other option is practical for the grower, then strips less than 6 rows should be allowed.

There are a number of pest management realities faced by corn growers in areas historically infested with heavy levels of SWCB that affect grower decisions. Economically, these farmers must be able to treat their refuges routinely with insecticides to avoid devastating losses (70 bu or more in untreated situations have been measured). From a practical standpoint, it is likely that these farmers will choose well-defined blocks that can be treated with insecticides over small, repetitive in-field strips of non-Bt corn that cannot effectively be treated without also paying for unneeded treatment of the Bt corn. Thus, to meet their refuge requirements, it is likely that most growers in areas heavily infested with SWCB will employ nearby fields as adjacent blocks.

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?

The Panel believed that, conceptually, IRM plans can be improved by providing different requirements for different field conditions. Practically, there will never be enough information to make meaningful IRM recommendations for all of the different field conditions that may arise.

When there are substantial, identifiable differences, some meaningful differentiation may be possible. Thus, the Panel concluded that a regional approach for the major target pests of corn is appropriate when differences are clear and consistent, and that judicious establishment of regional IRM working groups would be helpful in developing policies.

The main drawback with a regional approach is that the boundaries of the region can be perceived as arbitrary. To partially counter this perception, the Agency could require some educational effort to justify to farmers why the boundaries were chosen. Alternatively, the Agency could convene stakeholder groups of interested and affected parties near the potential boundaries to receive input on boundary choice. This approach has problems, but should not be dismissed because the potential benefits of getting grower buy-in and marginally improving the plan could be substantial.

All of the models and existing data suggest that the current IRM plan for Bt corn in the Midwest should be effective. Some Panel members felt that the 20% refuge may be too small in high insecticide use areas, because insecticide products with >90% efficacy are now available. This issue was briefly discussed in response to question 3 and will be addressed later in response to question 11. The areas with high insecticide use may include regions other than those with SWCB problems (e.g., parts of the irrigated region of Nebraska). It might be possible to define these areas on the basis of insecticide use alone. One Panel member commented that a scientific analysis of the effect of periodically spraying the refuge has not been completed, so it is not possible to support the sprayed refuge concept scientifically.

For CEW, the spatial arrangement of the refuge is not considered to be important because there is no high dose for this insect, and Bt resistance genes have been found in CEW that have partially dominant inheritance. What is important with CEW is providing a very large percentage of the acreage in an area-wide refuge, as was addressed in the Panel response to question 3. Therefore, in areas with both the ECB and CEW, the spatial arrangement of the refuge can be dictated by the biology of ECB, but the size of the overall refuge in a region should be dictated by the lack of high dose for CEW.

One Panel member indicated that while it may be appropriate to increase refuge requirements for the southern region, even a 50% pure refuge strategy will not accomplish the IRM goals.

The key pests for IRM in the United States are ECB, SWCB and CEW. CSB is an occasional pest of corn that is concentrated in field margins near borders with grassy vegetation. Generally growers do not try to spray for CEW, thus if resistance were to evolve in this species, the environmental and social cost is likely to be minimal.

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

The Panel concluded that frequent insecticide treatments are detrimental to resistance

management if only the refuge is treated. Conceptually, a 20% refuge policy with a highly efficacious insecticide (>90% kill) used in the refuge is equivalent to a 2% unsprayed refuge policy. If frequent insecticide treatments are always targeted at both refuge and Bt corn, the impact on resistance management will be negligible as long as the treatments are as equally effective on Bt-resistant and susceptible insects.

The Panel was divided regarding the best practical approach for establishing and managing IRM practices in areas that are heavily sprayed for target pests of Bt-corn. Some Panel members concluded that a greater than 20% sprayed refuge is justified, at least until more definitive work shows conclusively why such levels should not be considered. Others held that retaining the current uniform 20% sprayed or unsprayed refuge level throughout all non-cotton areas would result in better overall IRM compliance. An additional possibility is to restrict insecticide use on the refuge to those products that provide economic control, but <70% kill. It was noted that the level of market penetration within most of the heavily sprayed area is reported to be at or below 50% and that this may increase the effective size of the current refuge. However, the spatial scale at which the insecticide use or market penetration was based has not been defined scientifically. Although new insecticides can kill more than 90% of ECB, the point was made that in some of these areas, SWCB rather than ECB is the primary target. Since the two species frequently do not emerge exactly at the same time (Posler et al, 1991) and since there may be a narrow window of opportunity for achieving maximum control, the ECB population may realistically decline less than would be possible to achieve with perfectly timed insecticide applications.

The Panel noted that a subcommittee of the North Central Regional Research Project 205 (NC205) is considering the issue of sprayed refuges. The subcommittee of NC205 recognizes that more information regarding grower spray practices is needed before the question can be adequately addressed. The SAP did not address the issue of what was the appropriate spatial scale for making these determinations by NC205. In addition, the Panel expressed a desire for compliance to be verified through an independent (non-registrant) mechanism.

It was reemphasized that heavily sprayed areas do not define the historical geographical distribution of SWCB and that it was important to focus on the fact that the need or choice to employ corn borer insecticides (for whatever species and reason) at a time of corn borer vulnerability is what potentially compromises some of the resilience in the resistance management plan by causing population reductions in the refuge. Related to this observation, some researchers have proposed that another option might involve tying next year's refuge size to the current year's pesticide use usage. One concept proposes that if 20% refuges are treated during the current growing season, then larger refuges might be mandated on that farm during the next growing season unless certain mitigating circumstances develop. This idea seeks to establish an incentive for preserving corn borers in the refuge. These ideas have not yet been scientifically evaluated.

In some areas, pesticides are used for control of pests that are unaffected by Bt corn. In these circumstances, it is likely that both Bt and non-Bt corn will be treated equally, and the timing of these sprays may not coincide with ECB activity. In Nebraska, the best available

information derived from extension entomologists, grower surveys, and crop consultants is that 25% of the acreage receives an insecticide treatment for ECB. However, evaluation of insecticide use at finer spatial scales may reveal areas with much higher insecticide use. Many of the additional insecticide treatments are targeted at other pest species (e.g., western corn rootworm adults, western bean cutworm, and spider mites).

***Bt* Sweet Corn**

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?

The Panel agreed on the importance of recognizing that sweet corn represents only 1% of the total corn production nationally, but that it can make up a more substantial portion of the corn acreage in some regions of California and Florida.

The Agency does not currently mandate a refuge for sweet corn, but growers are required to destroy any stalks that remain in the fields within 30 days after harvest. The Panel believes that this strategy is sufficient. Furthermore, the simple strategy of destroying stalks which may house resistant ECB is not difficult for growers to institute, so high compliance is expected. The techniques used to destroy crop residue may vary depending on production region, tillage system and rotation practices. The most common technique that is available to growers is the rotary mower which shreds plant material into small pieces and kills most insects. Tillage following rotary mowing may result in some additional mortality, but can disrupt reduced tillage practices. In areas where double cropping occurs, rotary mowing and/or tillage for the second crop should be sufficient. Some Panel members recommended shortening the crop destruction period after harvest to 14 days if insects could emerge before 30 days. Other Panel members recognize that in some regions of the country, a delay in crop destruction should not matter if insect overwintering occurs in plant residues.

Compared to field corn, sweet corn will contribute very little to the development of resistance in pests such as ECB, CEW and fall armyworm (FAW), as long as stalks are destroyed. This is due to the limited acreage as well as the fact that corn ears are typically picked before ear infesting larvae can develop to the pupal stage. Whorl stage Bt sweet corn seems to provide a high dose for ECB and CEW, but not for FAW.

***Bt* Corn and *Bt* Cotton**

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?"

The Panel concluded that it did not have detailed information on the current monitoring programs. It was difficult for the Panel to comment on the adequacy of the current programs. The suggestion was made that there be a careful peer review to assess the adequacy of all Bt resistance monitoring programs, as was suggested for assessing modeling efforts.

It was generally agreed that monitoring will not remedy the faults of an ill-conceived IRM plan but that monitoring should be used to assess the success of the current plan. In general, the Panel felt that the appropriateness of different techniques that have been or are being used for monitoring resistance depends on the insect, crop, and the question(s) being asked. For example, the most appropriate technique for monitoring resistance in a pest for which a crop provides only a moderate dose is unlikely to be the best technique for monitoring a pest for which a crop provides a high dose. The most appropriate technique for an insect with an obligate diapause that can not easily be raised on artificial diet is probably going to be different than the technique appropriate for an insect with a four week generation time that is easily reared and mated in the laboratory.

If the question being asked is simply whether or not a pest population has become predominantly composed of resistant individuals, monitoring a single location would be straightforward. However, if the goal is to detect small increases in low resistance allele frequencies, carefully crafted experimental designs and statistical analyses are needed.

A major question discussed by the Panel was whether a discriminating or diagnostic dose would detect a change in resistance allele frequency before resistance to a high dose/refuge approach was too high for remediation. Most of the Panel felt that these approaches could, at best, detect resistance when the resistance allele frequency had reached 1%. Some Panel members believed that there could be utility in detecting resistance alleles at this frequency. One Panel member indicated that the use of surface applications of a toxin over artificial diet yields suspect results because larvae could crawl under the diet surface. Another comment addressed the potential of screening techniques that used plant material to have more false negatives than diet incorporation assays. These comments clearly suggest the need to carefully test biases in all monitoring methods before they become *de facto* standards.

A number of Panel members felt that in many cases, the F_2 screen accompanied by field screening could be very effective for detecting low frequencies of recessive and dominant resistance alleles. A number of articles have already been published on the economics and sensitivity of the F_2 and field screening techniques developed for ECB (Andow and Alstad, 1999, 1998; Andow et al., 2000, 1998). There is a need to validate the F_2 screen by use of lab colonies with known frequencies of resistance alleles. One such study has been completed by Zhao and Shelton (in press) using the F_2 screen with well characterized strains of the diamondback moth.

Their results indicate that the F_2 screen can document low allele frequencies, but using a Bt plant as the screening method on the F_2 generation may be inappropriate. Additional research is probably needed for each insect-crop system before the F2 screen is used in that system.

The Panel agreed that sampling efforts must be concentrated in areas of high risk. The issue of what constitutes an area of high risk needs to be considered thoroughly from a scientific perspective. A useful interim definition is a region with high usage of a Bt crop, where high usage could be determined based on the overall acreage of the crop planted in the area and the percentage of the crop that produced the Bt toxin.

The questions of how many areas of high risk should be sampled was difficult to answer, and the Panel felt that economic concerns in addition to science would dictate the answer. One Panel member explained that examination of the genetic differentiation of insect samples over large transects could help answer that question. Such studies were already available for some target pests (Korman et al., 1993 and Bourguet et al., 2000) and the results differed depending on the pest and the geographic area where the study was conducted. More work is needed to gather and analyze such data on other species, and in a number of other regions.

Comments were made that the relevance of the CBW "tolerance" described by Sumerford et al., (2000) should not be interpreted as a method to evaluate the effectiveness of the current refuge recommendations. It is preliminary and is limited in that it lacks the experimental design necessary to make strong scientific statements. However, it should not be completely dismissed. It should be seen as an indication that more research needs to be focused in "problem areas." The small sample sizes (which could bias the results either way, depending on the nature of the resistance trait) were the greatest concern in the data presented by Sumerford et al.

Regarding monitoring of the pink bollworm in the Southwest, a Panel member familiar with the situation indicated that the current sampling is adequate for detecting failures of Bt cotton in the various cotton-producing regions of Arizona. However, better statistical methods need to be developed. Bioassay-based monitoring is complemented by sampling of pairs of Bt and non-Bt field to monitor field efficacy throughout the areas in which Bt crops are grown. The PBW bioassay method and discriminating concentration have been validated (Simmons et al, 1998; Liu et al, 1999; Patin, 1999; Tabashnik et al, 1997; Carriere et al, in press).

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?

The Panel pointed out that in cases such as CBW and TBW, there were no remedial action plans in place so the question of improving them was premature. In one case, however, a detailed plan was in place (e.g., Arizona Rapid Response Team). The Panel concluded that in general, the Arizona Rapid Response Team approach was a good one for slowing the increase in resistance gene frequency and could be used as an approach in developing other such plans.
In the 1998 SAP report, the Panel suggested that regional working groups be formed, and that they develop such plans. There was disagreement on the Panel about the best design of a remedial action plan. One Panel member concluded that monitoring methods could not detect resistance early enough to implement remedial action.

An important question raised by another Panel member was whether the goal of the remediation plan is to slow the spread of the gene, or to eradicate the resistance gene. The remedial action plan will differ depending on the goal. The opinion was expressed that, for some insects, remedial action plans need to be implemented within one week if the goal is to eradicate the resistance gene. If implementation required greater than seven days, some individuals will likely enter pupation, thus providing the opportunity for the gene to migrate to other areas. Conducting a remedial action plan with a goal of resistance gene eradication will be extremely costly. Additionally, because of our limited ability to detect resistance on fine spatial scales, it may be impossible.

On a broad level, most of the Panel members concluded that a remedial plan to slow the rate of increase of resistance genes (and perhaps to cause their decline) needed to include the following:

1) Education of the grower and crop consultants to look for any changes in the level of control. Because of the number of growers involved in growing Bt plants and their high level of concern for their crops, they are probably going to be the first to detect changes in performance.

2) Monitoring for changes in plant damage, insect susceptibility and/or allele frequency is imperative. Any changes should be followed up in a rapid manner for verification. Once verified, growers, extension specialists, state and federal officials should be notified, and alternative strategies, appropriate for the area, should be implemented. If resistance is verified, the geographic area should be well-defined with further investigations. Once the area has been delineated, a regional registration should be pursued until further clarification and a remedial plan is put into place.

3) The registered product should not be returned to market in that region until the Agency has data to indicate that returning it to market would have more benefit than risk. Factors which should influence this decision would include the risks and benefits of alternative strategies (e.g., foliar insecticides, sterile insect release, cultural practices, etc.). Without an understanding of the stability of resistance, it is difficult to know from a biological standpoint if and when the product should be reintroduced into the region.

4) The success of the remedial action will be difficult to document. During the remedial action period, one would need to continue monitoring the pest population through assays as noted above. Additionally, it seems reasonable that blocks of isolated Bt crops should be planted in the region as a research tool.

5) As part of the remedial action plan, it is important to understand why resistance occurred. This needs to be done through better documentation of compliance efforts that growers undertook prior to the problem.

At a conceptual level, two types of remedial responses can be taken. The first approach is to reduce the selective advantage of the resistance allele by increasing mortality or reducing fecundity of resistant types in the Bt field. This can be done by reducing mortality or increasing fecundity of insects associated with the refuge. The second approach is to modify the mating system so that fewer resistance alleles are passed on to future generations. Because of logistical issues around confirming measurements and organizing a response, one Panel member felt that there is likely to be a 2-year time delay between detecting and measuring the resistance frequency and implementing a remedial response. During this time, increasing refuge size from 20% to 66% could prolong susceptibility 10 generations. In addition, decreasing survival and reproduction of moths from Bt corn fields by 90% is predicted, based on a genetic model, to prolong susceptibility by 10 generations (Andow and Ives). Modification of the mating system by changing movement rates and attracting susceptible males into Bt corn fields could prolong susceptibility for >20 generations. Although these results suggest that remedial actions could increase the durability of Bt corn, much work remains to prove that they can be effective management interventions.

A practical comment was that remedial action in the Southwest should not be implemented on a county-wide basis, as previously indicated in Agency documents, because doing so might result in illogical and possibly counter-productive remediation actions, and could penalize producers who have very effectively combated resistance on their farms by appropriate use of refuges.

Another comment was made specifically about the CBW/TBW situation in the cotton belt. Currently there are three proposed remedial action plans for CBW/TBW on Bt cotton. None are ready to be put forth as the prescribed remedial action plan.

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 System)? What compliance mechanisms (e.g., grower contracts, sales incentives, insurance) does the SAP believe will maximize compliance?

The Panel recognized the usefulness of grower surveys for assessing grower trends (e.g., Rice and Pilcher surveys), but generally agreed that they are not a reliable tool for assessing grower compliance with IRM. Basically the problem is that while growers who plant refuge and

treat it properly have no reason to misrepresent their actions, growers who do not comply with refuge requirements have good reason to misrepresent their actions. Surveys conducted to provide an accurate measurement of grower compliance with IRM requirements must be designed carefully to take these factors into account.

Several Panel members recommended that the surveys should be designed and conducted by independent parties. The Panel also suggests that second party surveys of groups that may have more accessible information related to pest management practices (e.g., crop consultants or seed and chemical dealers) could provide more reliable information related to grower practices. The Agency's reliance on the registrant companies to monitor grower compliance was seen as a major problem. Some Panel members recommended that the Agency should develop an independent compliance monitoring program.

Mechanisms that reduce the cost of compliance will be more effective at improving compliance. Currently, there is very little research available to use in assessing which mechanisms are most effective for IRM and which mechanisms are more costly to implement. One Panel member suggested that the mechanism that will be most effective combines grower education, with grower contracts, a refuge deposit and refund. For example, growers pay a deposit on Bt corn and receive a contract and educational materials explaining IRM requirements. To get their deposit refunded, growers must complete and sign the contract, which requires them to indicate where Bt seed was planted, where refuge seed was planted, and evidence of the purchase of non-Bt seed. If set appropriately, only noncompliant growers will choose to forfeit their deposit, which allows compliance monitoring efforts to be more focused. This mechanism, however, is likely to be more costly to implement than current education efforts and grower contracts. There is currently no research available to determine if it or any other approaches will result in a meaningful increase in compliance.

Use of the Global Positioning System to map grower transgenic and non-transgenic fields in a region was recognized as an important tool as long as it was coupled with grower interviews. Although this system could be useful for Arizona cotton, it is not yet practical for all regions of the country and for all production systems.

The Panel generally agreed that grower adoption of IRM plans is best done through education and grower contracts. The essential needs for IRM compliance and IRM plans should be continuously "advertised" through extension programs, pest control advisors, company literature, and dealer training programs. The benefits and importance of participation by individual growers must be emphasized and a compliance culture should be developed. Educating growers about why IRM plans are needed and how they are successfully implemented can increase compliance for growers who are unaware or confused by the requirements. It may also improve compliance rates among growers with a strong sense of stewardship. Education does not however reduce the cost of compliance to growers, so while education is likely to increase compliance, it will not ensure compliance.

Grower contracts can improve compliance using the threat of sanctions. If a grower is caught violating IRM guidelines, the grower risks losing the opportunity to use Bt plant-pesticides in the future. Two factors will influence the success of grower contracts: (1) the cost to growers of losing access to Bt plant-pesticides and (2) the likelihood that they are detected violating IRM requirements. As long as monitoring grower compliance is costly, extensive monitoring is not feasible and the likelihood of detection will be small. The smaller the likelihood of detection, the lower compliance rates will be.

Sales incentives can improve compliance by directly reducing the cost of compliance. If growers are given conventional seed to compensate them for the losses anticipated on the refuge, they have an incentive to plant refuges because otherwise they waste valuable seed. Since this type of sales incentive reduces the cost of compliance, it may improve compliance rates. A potential problem with this type of incentive is that growers can resell the seed and forgo planting refuge or may plant refuge in an inappropriate configuration. To ensure that a refuge is actually planted in an appropriate configuration, monitoring may still be required.

Refuge insurance provides incentives for growers to comply with refuge requirements because they receive an indemnity to compensate them for refuge losses when they have planted refuge in an appropriate configuration. This indemnity reduces the cost of compliance and will improve compliance rates. A problem with refuge insurance is implementation. To implement refuge insurance, a premium must be paid to cover expected losses and the administrative cost of adjusting losses. Mitchell et al. (2000) showed that growers will probably not be willing to pay a premium that is high enough to cover indemnities and administrative costs. If growers do not buy the insurance, they will not have the incentive to comply with refuge guidelines. Refuge insurance could work as a compliance mechanism only if it were made compulsory or the premiums were subsidized.

Refuge deposits and refunds provide an incentive for growers to comply with refuge recommendations by increasing the cost of noncompliance. For a refund and deposit mechanism, a grower pays a deposit in addition to the technology fee when purchasing Bt seed. The deposit is refunded when the grower provides evidence that the refuge was planted in the appropriate configuration. If the grower does not provide suitable evidence of compliance, the grower forfeits the deposit. Obstacles to implementing a deposit and rebate mechanism involve defining what constitutes suitable evidence of compliance with refuge requirements and what is the administrative cost.

REFERENCES

Andow, D.A., and Ives, A. Adaptive resistance management. , *Ecol Appl.*, (submitted).

Andow, D.A., et al., 2000. Frequency of resistance to *Bacillus thuringiensis* toxin Cry1Ab in an Iowa population of European corn borer (Lepidoptera: Crambidae), *J. Econ. Entomol.*, 93, 26.

Andow, D.A. and Alstad, D.N. 1999. Credibility interval for rare resistance allele frequencies, *J. Econ. Entomol.*, 92, 755.

Andow, D.A., et al. 1998. Using an F_2 screen to search for resistance alleles to *Bacillus thuringiensis* toxin in European corn borer, *J. Econ. Entomol.*, 91, 579.

Andow, D.A. and Alstad, D.N. 1998. The F_2 screen for rare resistance alleles, *J. Econ. Entomol.*, 91, 572.

Andow, D.A. and W.D. Hutchison. 1998. Bt-corn resistance management. *In:* M. Mellon and J. Rissler (eds.), Now or Never: Serious New Plans to Save a Natural Pest Control. Union of Concerned Scientists: Cambridge, MA, pp.19-66.

Bourguet, D. M.T. Bethenod, N. Pasteur, and F. Viard. Gene flow in the European corn borer, *Ostrinia nubilalis*: implications for sustainability of transgenic insecticidal maize. *Proc. Royal Soc. London* Series B: 267: 117-122.

Caprio, M. A., D. V. Sumerford & S. R. Sims. 2000. Evaluating transgenic plants for suitability in pest and resistance management programs. p. In press. In: L. Lacey & H. Kaya [eds.], Field Manual of Techniques for the Application and Evaluation of Entomopathogens. Kluwer Academic Press

Caprio, M. A. 1998. Evaluating resistance management strategies for multiple-toxins in the presence of external refuges. *J. Econ. Entomol*. 91: 1021-1031.

Carrière, Y., T.J. Dennehy, B. Pederson, S. Haller, C. Ellers-Kirk, L. Antilla, Y.- . Liy, E. Willot, and B.E. Tabashnik. Large-scale management of insect resistance to transgenic cotton in Arizona: can transgenic insecticidal crops be sustained? *J. Econ. Entomol. in press*

Hunt, T. E. 1999. Dispersal and behavior of adult European corn borer in and around corn. University of Nebraska Ph.D. dissertation.

Hurley, T.M., S. Secchi, B.A. Babcock, and R.L. Hellmich (in press). Managing the Risk of European Corn Borer Resistance to Bt Corn. The Economics of Modeling Environmental Impacts of Agricultural Biotechnologies (T. Swanson, ed.). Kluwer.

Hurley, Terrance M., Bruce A. Babcock, and Richard L. Hellmich. Bt crops and Insect Resistance: An Economic Assessment of Refuges. *Journal of Agricultural and Resource Economics* (in press).

Korman, AK; Mallet, J; Goodenough, JL; Graves, JB; Hayes, JL; Hendricks, DE; Luttrell, R; Pair, SD; Wall, M. 1993. Population structure in Heliothis virescens (Lepidoptera: Noctuidae):

an estimate of gene flow. Ann. Entomol. Soc. Amer 86, 182-188.

Liu, Y.-B., B.E. Tabashnik, T.J Dennehy, A.L. Patin, and A.C. Bartlett. 1999. Development time and resistance to Bt crops. *Nature, 400: 519.*

Mitchell, Paul D., Terrance M. Hurley, Bruce A. Babcock, and Richard L. Hellmich. 2000. Economic Evaluation of Bt Corn Refuge Insurance. CARD Working Paper 00-WP 243.

Onstad, D. W., and C. A. Guse. 1999. Economic analysis of transgenic maize and nontransgenic refuges for managing European corn borer (Lepidoptera: Pyralidae). *J. Econ. Entomol*. 92: 1256-1265.

Patin, A.L., T.J. Dennehy, M.A. Sims, B.E. Tabashnik, Y.B. Liu, L. Antilla, D. Gouge, T.J. Henneberry, and R. Staten. 1999. Status of pink bollworm susceptibility to Bt in Arizona. *Proc. Beltwide Cotton Conferences. National Cotton Council, pp. 991-996.*

Posler, S., M. Sandoval, and G. Dick. 1991. Corn borer moth flights in Finney County, Kansas. Kansas State Univ. Southwest Research-Extension Center 1991 Field Day Report. Report of Progress 630, p. 30.

Showers, W. B., R. L. Hellmich, M. E. Derrick-Robinson, M. E., and W. H. Hendrix III. Aggregation and dispersal behavior of marked and released European corn borer (Lepidoptera: Crambidae) adults. Submitted *J. Environ. Entomol.*

Simmons, A.L., T. J. Dennehy, B.E. Tabashnik, L. Antilla, A. Bartlett, D. Gouge, and R. Staten. 1998. Evaluation of Bt cotton deployment strategies and efficacy against pink bollworm in Arizona. *Proc 1998 Beltwide Cotton Conferences. 1025-1030.*

Stone, TB; Sims, SR. 1993. Geographic susceptibility of Heliothis virescens and Helicoverpa zea (Lepidoptera: Noctuidae) to Bacillus thuringiensis. *J. Econ. Entomol*. 86, 989-994.

Sumerford, D. V., W. L. Solomon, and D. D. Hardee. 2000. Heritable tolerances of Cry1Ac in Lepidoptera pests of cotton. Proc. Beltwide Cotton Prod. Res. Conf., pp. 1053-1055.

Tabashnik, B.E., A.L. Patin, T.J. Dennehy, Y.-B. Liu, Y. Carrière, M.A. Sims, and L. Antilla. Frequency of resistance to Bacillus thuringiensis in field populations of pink bollworm. *Proc. Natl. Acad. Sciences. 97:12980-12984.*

U.S. Environmental Protection Agency. 1998. The Environmental Protection Agency's White Paper on Bt Plant-Pesticide Resistance Management. Biopesticides and Pollution Prevention Division, Office of Pesticide Programs, Office of the Assistant Administrator for Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency, 401 M Street, SW,

Washington, DC 20460.

SAP Report No. 2000-07b, March 12, 2001

REPORT:
FIFRA Scientific Advisory Panel Meeting,
October 18-20, 2000, held at the Marriott Crystal City Hotel,
Arlington, Virginia

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Bt Plant-Pesticides Risk and Benefit Assessments: Gene Flow/Outcrossing, Environmental Fate in the Soil and Non-Target Organism Effects

Mr. Paul Lewis
Designated Federal Official
FIFRA Scientific Advisory Panel
Date:_____

Christopher Portier, Ph.D.
FIFRA SAP Session Chair
FIFRA Scientific Advisory Panel
Date:_____

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
October 18-20, 2000

Bt Plant-Pesticides Risk and Benefit Assessments: Gene Flow/Outcrossing, Environmental Fate in the Soil and Non-Target Organism Effects

PARTICIPANTS

FIFRA SAP Session Chair
Christopher Portier, Ph.D., National Institute of Environmental Health Sciences, Research Triangle Park, NC

FIFRA Scientific Advisory Panel
Stephen Roberts, Ph.D., University of Florida, Gainesville, FL

FQPA Science Review Board Members
David Andow, Ph.D., Department of Entomology, University of Minnesota, St. Paul, MN
Mitch Cruzan, Ph.D., Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN
Brian Federici, Ph.D., University of California, Riverside, Department of Entomology and Graduate Programs in Genetics and Microbiology, Riverside, CA
Richard Hellmich, Ph.D., USDA, ARS Corn Insects and Crop Genetics Research Unit Ames, Iowa
Eliot Mark Herman, Ph.D., United States Department of Agriculture, Agricultural Research Service, Beltsville, MD
Randy Higgins, Ph.D., Kansas State University, Department of Entomology, Manhattan, KS
Rosalind James, Ph.D., USDA, Kika De La Garza Subtropical Agricultural Research Center Weslaco, TX
Stephen B. Malcolm, Ph.D. , Department of Biological Sciences, Western Michigan University, Kalamazoo, MI
Robert V. Miller, Ph. D., Oklahoma State University, Department of Microbiology and Molecular Genetics, Stillwater, Oklahoma
Karen Oberhauser, Ph.D., Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN
Ian L. Pepper, Ph.D., Department of Soil, Water and Environmental Science, University of Arizona, Tucson, AZ
John Pleasants, Ph.D., Department of Zoology and Genetics, Iowa State University, Ames, Iowa
Alison G. Power, Ph.D., Department of Ecology & Evolutionary Biology, Cornell University, Ithaca, NY
Mark Sears, Ph.D., University of Guelph, Department of Environmental Biology, Guelph, Ontario, Canada
Blair Siegfried, Ph.D., Department of Entomology, University of Nebraska, Lincoln, NE

Charles Neal Stewart, Jr., Ph.D., University of North Carolina at Greensboro, Department of Biology, Greensboro, NC
Guenther Stotzky, Ph.D., Department of Biology, New York University, New York, NY
Chip Taylor, Ph.D., Department of Entomology, University of Kansas, Lawrence KS
John H. Willis, Ph.D., Department of Biology, Duke University, Durham NC

Designated Federal Official
Mr. Paul Lewis, FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, Environmental Protection Agency, Washington, DC

PUBLIC COMMENTERS

Oral statements were made by:
Mr. Graham Head, on behalf of Monsanto
Ms. Janet Carpenter, on behalf of the National Center for Food and Agricultural Policy
Ms. Demetra Vlachos, on behalf of Novartis Seeds
Margaret Mellon, Ph.D., on behalf of the Union of Concerned Scientists
Val Giddings, Ph.D. on behalf of the Biotechnology Industry Organization
Mr. Eric Sachs, on behalf of ABS Technical Committee
Dennis Calvin, Ph.D., on behalf of Pennsylvania State University
Rebecca Goldburg, Ph.D., on behalf of Environmental Defense
Lincoln Brower, Ph.D., on behalf of Sweet Briar College
James White, Ph.D., on behalf of USDA, Animal Plant Health Inspection Service
H.B. Skip Mattews, Ph.D., on behalf of NIEHS

Written statements were received from:
Agricultural Biotechnology Stewardship Technical Committee
Auburn University
Aventis CropScience
Council for Agricultural Science and Technology
Dow Agrosciences, Inc.
Monsanto
National Cotton Council
Novartis Seeds, Inc.
Pioneer Hi-Bred Inc.
Union of Concerned Scientists

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency pertaining to Bt Plant-Pesticides Risk and Benefits Assessment: Gene Flow/Outcrossing,

Environmental Fate in the Soil and Non-target Organism Effect). Insect Resistance Management. Advance notice of the meeting was published in the *Federal Register* on September 6, 2000. The review was conducted in an open Panel meeting held in Arlington, Virginia, on October 19, 2000. The meeting was chaired by Christopher Portier, Ph.D. Mr. Paul Lewis served as the Designated Federal Official. Douglas Gurian-Sherman, Ph.D. (EPA, Office of Pesticide Programs) discussed gene flow, outcrossing and environmental fate in the soil. Zigfridas Vaituzis, Ph.D. (EPA, Office of Pesticide Programs) discussed non-target organism effects.

In preparing this report, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This report addresses the information provided and presented within the structure of the charge by the Agency.

CHARGE

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?

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?

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.

DETAILED RESPONSE TO THE CHARGE

The specific issues to be addressed by the Panel are keyed to the Agency's background document "Preliminary Risk and Benefit Assessment for Bt Plant-Pesticides," dated September 20, 2000, and are presented as follows:

Gene Flow/Outcossing Questions

General Comment

The SAP applauds the EPA for their forethought in the future of risk assessment by including questions 1 and 2 in their generalized format and utilizing this opportunity to gather information to help make future informed decisions.

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 US? If yes, what further risk assessment is warranted to evaluate the risk of outcrossing?

The Panel began its response by presenting several definitions as relevant to the question. *Hybridization* is cross fertilization and the production of viable offspring by mating between two divergent lineages (i.e., strains, races, ecotypes, varieties, subspecies, or species) that have remained isolated long enough to develop distinctive sets of morphological, physiological, or genetic traits. In this context, crossing between normal and transgenic strains constitutes hybridization.

Introgression is gene flow between divergent lineages via hybridization. For introgression to occur, the production of viable and fertile F_1 (first generation) and backcross hybrids is necessary. Transfer of genes from one lineage to another progresses through recursive crossing between advanced backcross hybrid generations and one of the parental strains.

Outcrossing is cross-fertilization among individuals within a strain (as opposed to selfing). The frequency of outcrossing is determined by morphological and physiological characteristics that facilitate fertilization by outcrossing pollen over self pollen.

In response to this question, the issue of quantification of hybridization and introgression rates provide a first tier assessment and could answer the question of risk if there are no wild relatives in proximity to crop cultivation. In most cases where wild relatives co-occur with transgenic crops, some gene flow would be expected even at substantial distances. If hybridization and introgression do occur, then another tier of risk assessment is needed, e.g., the consequences of plant/transgene combinations in managed and unmanaged ecosystems. Even if gene flow is low (<1%), it may result in evolutionary changes in recipient species if selection favors the new trait. In many cases of protein plant-pesticides, there are few relevant data on gene flow and the strength of selection exerted by the herbivores or pathogens targeted by the pesticidal trait.

Hybridization and introgression in themselves do not necessarily equate to increased risk, *per se*. The likelihood that a transgene will persist and have any effects depends, in part, on gene flow rates and transgene expression in a wild relative. There are potential consequences of Bt transgenes: increased fitness, increased invasiveness and weediness. The biology of wild relative

plants needs more general study, as will be noted in subsequent answers to the questions below.

There are several necessary components for transgene escape. Transfer of pollen via biotic and abiotic vectors is the primary path of escape for nuclear transgenes. Considerations for risk include vector behavior and pollen longevity. Abiotic (wind) vectors produce relatively predictable pollen dispersal functions with grain density decreasing in proportion to the cube of distance from the source. Animal vectors (e.g., bees) are much less predictable and have been observed to fly considerably more than 100 m between sequential flower visits. Independent of the vector, a significant risk exists for low levels of pollen transfer over relatively long distances. For example, even for pollen with a short longevity, strong winds could transfer viable pollen a kilometer within a few minutes.

F_1 hybrids must be formed. First generation hybrids may pose minimal threat if they have low vigor or are infertile. However, even infertile hybrids could pose a threat if they are able to reproduce asexually. The production of fertile F_1 hybrids would create a genetic bridge between lineages that would promote introgression. Introgressive hybridization can occur when transfer of transgenes from one lineage to another requires the establishment of fertile F_1's and backcross hybrids. Finally, there is a chance for polyploid speciation. The production of fertile F_1 hybrids between normally incompatible lineages is possible via chromosome duplication after fertilization. Such polyploid species are fully fertile, but they would be reproductively isolated from the parental strains. If the new lineages were relatively vigorous, they could spread and establish new populations.

2. Are isolation distances as proposed for certified or registered seed considered 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?

The Panel concluded that there is limited probability of outcrossing from Bt-corn to weedy relatives because there are none to which Bt-corn can outcross to. However, adequate isolation distances for the protection of pure-strain crops (e.g. certified seed crop) may not be sufficient to ensure against transgene escape via hybridization. Additional measures need to be taken to establish buffer regions, pollen vector barriers, and larger regions that are free of close relatives. In cases where close relatives exist, monitoring programs should be implemented as will be described below.

Certified or registered seed isolation distances were designed to prevent crop-to-crop gene flow. The application at hand is crop-to-weed gene flow. Crops are generally self-pollinating, while weeds and wild relatives are often obligate outcrossers. Self-fertilizing crops may produce and disperse substantial amounts of pollen, but that pollen would tend not to result in outcrossing or gene flow between crop varieties at a given distance because of the selfing mating system. In contrast, that same pollen could be far more likely to result in gene flow, given the same distance, between the crop and its outcrossing wild relatives. Contamination rates between Bt-crops and their wild or feral relatives may therefore be substantially higher than that expected based on

isolation distances proposed for certified or registered seed. If the potential gene recipient species that are likely to receive the gene are in a large extant population, then gene flow will occur less than if the potential recipients are surrounded by interspecific, transgenic, donor plants. Therefore, many aspects of required isolation distances depend on the genetics and breeding strategies of the crop/weed system. The distances should be determined on a case-by-case basis.

Furthermore, even if gene flow within crops and between crops and relatives were the same, then typically, the isolation distances used for certified or registered seed allows some contamination from other cultivars. For example, Arizona cotton seed is allowed 0.08% contamination for certified seed, 0.05% for registered seed, and 0.01% for foundation seed. Isolation distances for different species of cotton are 660 ft for certified seed, 1320 ft for registered seed, and 2,640 ft for foundation seed in the absence of buffer rows. However, the isolation distance for cottons of different lint colors is three miles with no border or buffer fields. Some contamination is obviously expected to occur at the lesser distances for transgenic cotton. It would seem prudent to use, **as a minimum**, the distances required to contain color contamination. In general, isolation distances for transgenic crops should be substantially greater than those required for certified and registered seed and these are likely to be much greater for animal versus wind pollinated crops.

It should also be noted that isolation distances for certified seed might be inadequate for transgenic seed production. If the isolation distances allow for some contamination, then the transgene might spread to nontransgenic varieties grown for seed. These seeds would then be planted and sold with no consideration for the possibility of introgression because no one would know they contained the transgene.

Before one can prescribe isolation distances, more information is needed on the natural history, ecology, physiology, and genetics of the wild relative. If we can be reasonably certain that gene flow would not impact the invasiveness and weediness of the feral plant, then the recommended isolation distances might be sufficient for the F_1. Even then, it would be difficult to pinpoint individual weedy parents at times. However, rare hybridization events can be ecologically important—even a single event. If a transgene could allow the wild relative to increase in fitness or invasiveness, then greater stringency should be useful to gene containment. Technologies that mitigate gene expression, fertility, and gene flow (e.g., terminator) should be useful to gene containment.

Monitoring gene flow and persistence should be performed to determine the success or failure when the consequence of a gene could be large in a weedy wild relative. For example, green fluorescent proteins (GFP) or other molecular or morphological markers might be useful as monitoring tools. While it might be expensive, border areas of fields that contain transgenic crops should be monitored on a regular basis. In addition, broader surveys of surrounding regions (within 10 miles) would be made periodically (at least once a season) to monitor for hybridization as a result of rare long-distance pollen transfer.

3. Does the panel agree that the gene flow and outcrossing assessment contained in the background documents 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?

As note in response to question 2, corn and potato seem to be nearly risk-free with regards to gene flow in the USA. However, more documentation would have been desirable for potato. For cotton, the EPA should be commended for prohibiting the sale of Bt cotton in southern Florida and Hawaii, a precaution that is clearly warranted by the biological information presented in the EPA assessment. However, seed increase at commercial nurseries, etc., should also be reviewed relevant to gene flow considerations.

The Panel did not agree with the Agency's methodology for delimiting gene flow from commercial Bt cotton in seed nurseries to wild cottons that are endemic to Hawaii. There are several reasons for this conclusion.

First, little biological data were presented for the wild cottons. What are the particular insect pollinators? Are there potential herbivores in wild cottons that could be affected by Bt? What are their spatial distributions? What do we know about factors that limit population growth in the wild species? This information should be collected to develop a clearer understanding of hybridization and introgression of Bt transgenes into wild Hawaiian cottons.

Second, some evidence indicates hybridization and introgression between cultivated and wild cottons occur in Hawaii. What new requirement could mitigate transgene flow from Bt-cotton to wild cotton?

The isolation distances recommended for cotton may not be sufficient for bee-pollinated species. Also, the current EPA statement on IIC10 is odd—that 12 border rows of non-cotton should surround Bt cotton. It would seem to be more efficacious to include a pollen trap of non-Bt cotton. There would still be a high probability of gene escape—even if the cotton is used in the trap. It may be appropriate to limit the use of Bt cotton in Hawaii for the only registered uses, test plots and breeding nurseries (no commercial uses are allowed in Hawaii), until more is known about the ecology and genetics of the wild cottons in Hawaii and the consequence of Bt transgene expression in the wild relatives.

Currently there is no current restriction for cotton on the U.S. Virgin Islands, despite the fact that wild cottons probably exist there. While cultivated cotton is not currently grown there on a commercial scale, the Agency should consider cotton restrictions due to the concern of gene flow between cultivated and wild cotton.

Finally, several Panel members concluded that the U.S. needs to be a steward of international germplasm resources such as the teosintes that are native south of the USA border. The cultivation of Bt crops across US borders will certainly occur and the Agency should at least

have an understanding of its consequences.

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?

Various reports from both the SAP and the National Research Council have emphasized that potential risks associated with plant-pesticides need to be assessed on a case-by-case basis. While the classes and kinds of risks may be expected to be similar, risks for each new product must be characterized using data on the specific host species, the transgene, and the environment in which the product will be used. The Panel agrees with this general overview of risk assessment. It addresses several consequences for the development of a risk assessment for plant-pesticides.

First, it suggests that risk assessment processes and goals will be adjusted as information and experience accumulate. It is critical that the Agency have procedures in place to adaptively adjust the risk assessment process in a proactive way. Second, a commitment to a case-by-case analysis means that EPA risk analyses must either evaluate and justify the use of standard risk assessment tools or develop new models appropriate to the product under consideration. One Panel member indicated that the present analysis does not provide a justification for the use of the tiered toxicology model for any of the non-target evaluations. Third, the Agency should explicitly conduct both hazard identification and the identification of potential exposure pathways as the first step in risk identification. The Agency's non-target analysis assumes that a tiered toxin risk assessment is appropriate. The product, however, is a crop plant producing a protein toxic to invertebrates, so a broader examination of potential hazards and exposures should be done. To conduct this analysis, the Agency should require data on the expression of toxin in all major plant tissues. Fourth, after the hazard and exposure identification process, the Agency should consider which of these identified potential risks merit additional analysis by the applicant. After using this criterion to identify potential risks, the Agency can determine through a deliberative process which risks must be characterized and which would be supplemental, but not necessary. Fifth, to adequately address these issues on a case-by-case basis, the Agency will need additional staff and research capacities.

Applying these criteria to the Bt products under consideration, it appears to the Panel that collembola and earthworms are probably not appropriate indicator species for testing effects on soil invertebrates for these products. Bt Cry proteins have been tested against a wide variety of terrestrial and aquatic invertebrates including earthworms, collembola, daphnids, insect predators and parasites, spiders, and honeybees and have been shown to have a high degree of safety for these non-target organisms. In most cases, no adverse effects were observed even though test

populations were exposed to levels of toxin in excess of 500-1,000-fold higher concentrations than they would be expected to encounter under field conditions. The lack of any demonstrated adverse effects on populations of these organisms tested in the laboratory is due to a combination of selective traits that characterize Cry proteins, principal among these being that specific binding of these proteins to midgut microvilli is required for toxicity. In addition to laboratory studies, there are now a limited number of studies published in the literature showing that Bt crops also exhibit no direct adverse effects on these organisms under operational field conditions. These results are consistent with earlier studies of Bt strains used in bacterial insecticides, which demonstrated no significant detrimental impacts on populations of the non-target organisms studied noted above. While more studies on the effects of Bt-crops on non-target organisms under field conditions should be carried out to continue to assess the long-term effects of these crops on populations of non-target invertebrates, results of studies published to date indicate that Bt-crops should have no detrimental impact on non-target invertebrate populations, with the possible exception of certain species of insects belonging to the same order as the target insect.

On an individual basis, most of the Cry proteins reported to date generally have a narrow target spectrum, being toxic to either lepidopterous, dipterous, or coleopterous insects. Therefore, if a Cry protein is known to be toxic to one or more lepidopteran species, its likely spectrum of activity will be limited almost exclusively to other lepidopterans, but by no means will it be toxic to all or even to most of these. While non-target tests should continue to be carried out against other non-target invertebrates in the case of new Cry proteins, and against non-target endangered species in the same order of the target insect, results of these tests must be related to probable exposure of the non-targets under field conditions. Moreover, these results should be considered in a risk-benefit context by comparing the effects of the Bt crop on non-target invertebrate populations with those that would result from the use of registered synthetic chemical insecticides to control the target pest.

It is not clear that there would be any benefit to including additional soil invertebrates as potential indicator species. So how could one decide what species, if any to test? First, one would want to identify the particular risks that the specific product could cause to non-targets. Second, one would want to choose a species that might be affected by the toxin directly. Third, the organism should be in the target ecosystem, and fourth, it should be a dominant or important species in association with the particular risk.

Terrestrial isopods such as the common pillbug, *Armadillidum vulgare*, a detritovore, could be included in non-target studies, but experience to date and our knowledge of Cry protein molecular biology would argue against this, as there are unlikely to be any adverse effects on pillbug populations tested in the laboratory, or in the field for that matter. The same would be true for most other non-target invertebrates. With respect to using non-target coleopterans as indicator species to assess the risks of Cry proteins, the value of such indicators for the purpose of risk assessment is questionable. Species of beetles are the insects that may be directly affected, although this does not necessarily mean that other species will not be affected. Among the beetles, the common ground predators are carabids, tenebrionids, and staphylinids. Many beetles

species are also important participants in decomposition processes and may be sensitive to Cry3 proteins.

Data on at least some of the dominant species in the beetle groups mentioned above would be useful for cataloging the spectrum of activity of Cry proteins and for providing insights into the types of studies that might be done to examine food chain effects resulting from the use of Cry crops. On the one hand, it might be useful to know the sensitivity of a range of soil coleopterans to each Cry3 protein used in a Bt crop. But if one or a few such species, for example predatory carabids, a root feeding scarab, and a detrivorous staphylinid were tested in high-dose laboratory bioassays and shown to be sensitive to a particular Cry protein - a possible if not probable outcome - does this represent a risk from the Bt crop?

If such indicator species become required, it would appear that for the assessment of Cry proteins, it is inconsistent with requirements for synthetic chemical insecticides because equivalent indicators are not required for evaluating these insecticides, many of which are highly toxic to target and non-target coleopterans and other soil invertebrates and microorganisms. Such considerations need to be addressed for future data requirements.

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.

The Panel members concluded that data in the literature on Cry protein degradation, particularly under operational field conditions, were not adequate to address the issue of persistence of Cry proteins in Bt crop soil. Several laboratory studies have been conducted, but the relevance of these to growing Bt crops in actual field conditions is not known, as noted in response to question 4. With millions of acres of Bt corn and Bt cotton being planted in the U.S. in a variety of soil types and under various climatic conditions, most Panel members thought that excellent opportunities now exist for determining rates of degradation directly under field conditions. Despite the lack of data on the degradation of Cry proteins in soil, the Panel concluded that such data may not be necessary to conduct a preliminary risk assessment, although they may become necessary in conducting a final risk assessment. The Panel's basis for this decision is that historical data on Bt Cry proteins in bacterial insecticides have revealed no known adverse environmental effects in the soil, but information about the effects of Cry proteins delivered into the environment by Bt crops remains incomplete.

The Panel considered the key relevant soil degradation factors that should be addressed to be how long biologically active Cry proteins have accumulated and persisted in crop soils under field conditions and what are the adverse or beneficial consequences on soil organisms, including microorganisms. Most Panel members concluded that these questions were primarily of academic interest, but all agreed that such studies should be pursued to improve the knowledge base of

environmental impacts resulting from the application of Bt crops.

As background for these studies, there was some discussion of the laboratory studies that have been done and the different concepts of "half-life" used to quantify the degradation-persistence of Cry proteins. The "half-life" concept as applied in physics, for example, to quantify the decay of radioactive materials, was considered inapplicable to Bt proteins, which in soil would be subject to inherent and external physical as well as biological processes, e.g., enzymatic degradation. Thus, it should be realized that many studies on Bt Cry protein degradation, including those evaluated by the Agency, use the term "half-life" to mean the period for which 50% of the protein can be detected or is biologically active as determined through bioassays. In the case of radioactive materials, decay rates are expressed as an exponential function:

$$\text{decay rate} = e^{(-\lambda t)},$$

where lambda (λ) is the "decay constant". The decay constant can be calculated by fitting a line to a log transformation of the decay data. Bt decay rates do not appear to be exponential. Panel members differed whether using the radio decay model is appropriate for determining and describing the degradation of complex organics, such as Cry proteins in soil. Clearly, however, none of the data fits the exponential decay rate function. This is well documented by the wide variations for published half-lives ranging from 0.5 to 46 days for different Cry proteins from different laboratories. This wide variation in values is likely due to a combination of factors, including the inherent properties of the Cry protein tested, the conditions of the tests, and the statistical concepts employed to evaluate the data. Such wide variation makes it virtually impossible to use these data for risk assessment, thereby demonstrating the need for some kind of standardization of decay models, half-life concepts, testing procedures, and statistical analyses to quantify and evaluate Cry protein persistence in different soils. Moreover, in order to calculate half-life values, the kinetics of degradation (decay) must follow first-order kinetics. Published data on the degradation of Cry1Ab in soil do not follow first-order kinetics.

Even if first-order kinetics reasonably describe the decay rates of Bt proteins, the proteins would be present, in many cases, for a long time in soil. Furthermore, in the manner that the half-life concept has been used with the Cry proteins, the persistence of the proteins is dependent on the concentration of protein added. For example, with a half-life of 50 days (which is close to the 46 days reported by some investigators), the amount of protein remaining from the addition of 100 ng of protein/g of soil after six half-lives (i.e., 300 days) would be 1.56 ng/g of soil (which may still be toxic), whereas if the initial addition was 1000 ng of protein/g of soil, the amount of protein remaining after six half-lives would be 15.63 ng/g of soil (which would, based on published studies, be toxic). Hence, the application of the half-life concept to the persistence of Cry proteins in soil is dependent on knowing the level of protein being incorporated into the soil before it has much meaning from a biological viewpoint.

Whichever model is used to determine persistence in the soil, a concern of the Panel was the types of media and environments that have been used in the experiments to determine decay rates. These experiments have been carried out in experimental media such as various synthetic

media, sterile soil, and nonsterile soil. The protein was detected in natural soils throughout the growth of Bt corn and for months after harvest. Moreover, the protein was released in root exudates and from biomass of Bt corn and has been detected in natural soils both in a plant-growth room and in the field for 120 to 180 days, the longest time evaluated (Saxena and Stozky, unpublished). There are clear indications from the data available that various environmental factors can substantially affect biologically active lifetimes in soils. Based primarily on laboratory and plant-growth room studies, attachment to clays and humic matter have been shown to be able both to protect and alter bioavailability of Cry proteins in soil. pH and other basic environmental factors can also greatly affect the longevity of Cry proteins in soils, as shown in laboratory studies (Tapp and Stotzky, 1998). The presence of the soil microbiota appear to be important in the elimination of the protein. Stotzky (2000) observed that the soil microbiota was present in all natural soils in which persistence has been studied, and the microbiota was obviously present in the rhizosphere soil of corn, carrot, radish, and turnip in studies of the uptake of the protein from soil (Saxena and Stotzky, unpublished). Variations in the natural microbial community may significantly affect *in situ* half-lives of these proteins. Thus, there is a need for long-term field studies, as described in response to question 4.

The laboratory studies noted above indicate that data are needed to determine the extent to which Bt Cry proteins persist in soils under field conditions and whether persistence has adverse or beneficial effects on soil organisms. Thus, the long-term persistence of these proteins in soil, both after subsequent planting of Bt corn and of rotational crops, should be monitored in the field. Only a portion of Bt plants are harvested, and the remainder of the biomass is incorporated into soil. The protein may be totally degraded in the decaying biomass by microorganisms or may be released from disintegrating biomass and be bound on surface-active particles in soil, which protects the protein against biodegradation (Stotzky, 2000). The protein is also be released in root exudates throughout the growth of the Bt corn plants (Saxena and Stotzky, 2000; Saxena et al., 1999). Nevertheless, it must be emphasized that accumulation and even long-term persistence of Cry proteins in soil does not necessarily result in adverse environmental effects.

One Panel member felt that studies are needed that not only monitor persistence (e.g., degradation in litter-bags buried in and placed on the surface of soil), but that also measure vertical and horizontal migration of the toxins, especially to ground and surface waters. Such studies should include soils with different physicochemical characteristics (e.g., texture, mineralogical and organic matter composition, pH) and exposure to different environmental conditions (e.g., rainfall, depth to water table, tillage). These studies are important to assess long-term environmental effects of Bt-containing crops. Because currently available data do not demonstrate hazards of accumulated Cry proteins in soil, most members of the Panel did not believe that these studies should delay the Agency in preparing its risk assessment. Risk assessment is always a dynamic process, and when data from field studies become available, they should be evaluated to determine if they have a significant impact on the risk of continued use of these plants in agriculture.

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?

The Panel agreed that for the purpose of risk assessment, the principal issues are the amount of Cry protein that enters the soil over time and the length of time that the protein persists in a biologically-active form. These are also noted in response to questions 2 and 4. The mechanisms by which Cry proteins enter the soil, for example by secretion, shedding of root hairs, degradation of biomass pollen, etc., were to be considered of secondary importance, and although relevant, knowledge of these might not be necessary for completing a risk assessment. Nevertheless, as noted previously, knowledge of the biological mechanisms of the release of Cry proteins would be useful to understanding the fate of these proteins in the environment and their effects, positive or negative, on non-target organisms.

According to some plant physiologists, corn does not require a signal peptide to release proteins from roots. This is a characteristic apparently unique to corn. Studies are needed with Bt crops to determine whether Cry proteins are released from their roots, as this represents a continuous input of toxin into soil. Although not required by EPA, scientists in academia, government, and industry, should elucidate Cry release mechanisms including such studies as the effects of using different promoters, *cry* gene expression in different crops under different physiological conditions, and at different developmental stages, as well as Cry trafficking through plant tissues to the root surface. A variety of molecular, biological, and analytical techniques are now available that would make such studies rather routine, such as the use of green fluorescent protein (GFP) to monitor synthesis, expression level, secretion and accumulation in soil. Thus, whereas knowledge of secretion mechanisms may not be necessary to conduct a preliminary risk assessment, such knowledge may become necessary in conducting a final risk assessment.

In any case, current methods have demonstrated that the Cry1Ab protein is released from the roots of Bt corn throughout plant growth (Saxena and Stotzky, 2000; Saxena et al., 1999). For example, studies in hydroponic culture and in soil in a plant-growth room and in the field have demonstrated that Cry proteins are released in root exudates. In addition, during root elongation in soil, damage to root hairs and cells occurs, and this probably contributes to the amount of protein released into the soil. Thus, these studies indicate that Cry1Ab protein is likely to be present in the rhizosphere soil not only throughout the growth of the crop, but perhaps long after the crop is harvested. Methods for rhizosphere studies have historically been inadequate. The complex nature of the rhizosphere has made it difficult to simulate the soil-plant-microorganism continuum. Conversely, when attempts are made to simplify the system, the interactive nature of the ecosystem is lost, and it no longer is a true rhizosphere. Therefore, although distinguishing between active secretion and passive leakage may be important, aside from being difficult to evaluate, it may not be necessary for conducting a risk assessment. The more important aspect of this problem is to assume that continuous exposure to Cry proteins is likely within the soil system. For the purpose of risk assessment, these levels could be measured through a combination of

immunological assays for detection and biological assays to assess residual toxicity over time using sensitive indicator species.

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.

The data available on the accumulation of Cry proteins in crop soil is limited, especially with respect to published studies actually performed on assessing the accumulation of Cry proteins in soils where Bt crops have been grown in the field. In general, the Panel concluded that although not necessary for the Agency to complete a preliminary risk assessment, it would be prudent to determine under operational field conditions in different geographical regions and soil types, the extent to which Cry proteins accumulate in soil. Some Panel members recommended long-term field studies in soils with different physicochemical characteristics, under a variety of environmental conditions, and with biomass of different Bt crops both dicotyledonous and monocotyledonous. The Panel also proposed that these studies should extend over several cropping cycles, both with continuous cultivation of the Bt crops and with intermittent cultivation of rotational crops.

There have been several laboratory and plant-growth studies in which soil and soil components or soil microcosms with Bt Cry proteins or Bt crops were used in an attempt to provide insight into the fate of Bt proteins in crop soils. Superficially, these studies might appear to be contradictory, some showing accumulations that persist for over 200 days, whereas others show that most toxin is rapidly degraded within 7-14 days. However, the markedly different methods used to assess persistence most likely account for these differences. These differences include whether the experiment was done in sterile or non-sterile soils, whether it was done in field soils or not, whether the toxin used was the parasporal crystals found in Bt or the toxin that occurs in the Bt crop plants, and how toxin persistence was characterized (detection time versus "half-life"). There is disagreement among the committee members as to the relative importance of these various differences, so the scientific dimensions of these issues are outlined below.

Before briefly reviewing the more recent studies, it is worthwhile to note the principal findings of studies on persistence that were carried out with Bt spores and parasporal crystals more than 15 years ago (Pruett *et al*. 1980; West and Burges, 1982; West *et al*.,1984; West and Burges, 1985). The studies with parasporal crystals are relevant because these crystals are arguably the most chemically stable form of Cry proteins, whereas the proteins produced in Bt crops, which do not crystallize in plants, and therefore are not protected from proteolytic degradation, are the least stable form. In a relevant study, West and Burges (1985) showed that parasporal crystals mixed with grass in a soil microcosm had a “half-life,” as determined by bioassays, of only 9.5 days. The researchers attributed this rapid decline to microbial degradation. In contrast, they found that crystals remained toxic for more than two years when stored in sterile soil where they were not subject to microbial degradation (West *et al*., 1984). These results show

that parasporal crystals degrade rapidly when exposed to active microbial degradation and persist for a long time when not exposed to microbes.

More recent studies using purified Cry proteins or Bt crops in nonsterile soil microcosms in essence confirm this basic pattern. When bound to soils or soil components, Cry proteins remain toxic for periods of more than eight months (Tapp and Stotzky, 1998). Purified Cry1Ab protein, for example, was detected 234 days (the longest time studied) after addition to nonsterile soil microcosms maintained at optimal conditions of soil water tension and temperature. The same protein released from corn in root exudates was detected in soil after 120 and 180 days (the longest times tested) after plant harvest (Saxena and Stotzky, unpublished). In other laboratory studies or simulated microcosms, Cry1Ab was detected from 9 months (in litter-bags containing chopped Bt corn biomass) up to 17 months (in soil). Initial studies indicate that some of the activated Cry protein released from Bt plants into soil can avoid rapid degradation by microbes by binding to soils and soil components. In contrast, other studies using either purified Cry proteins or Bt crops in soil microcosms that included microbial communities showed "half-lives" from several days up to several weeks. For example, Cry2A cotton toxicity in a soil microcosm had a half-life of only 15-30 days (Sims and Ream, 1997). In another study, very little Cry toxin was found in soil combined with Bt cotton (Cry1Ac) after 140 days (Palm et al., 1996). The levels of Cry proteins remaining in the soil after a few weeks are typically well below 50 ng/g of soil, whereas the levels tested against non-target organisms in which no adverse effects were observed were more than 1,000 fold higher. These recent results suggest that toxin degradation rates may vary with the crop, the toxin produced by the transformation event, the microbial community in the recipient soil microcosm, the statistical characterization of toxin persistence, and/or the initial dose of toxin in the experiment.

Analysis of these studies suggest that under field conditions, Cry proteins in Bt crops will be degraded to minimal levels within a few or many months after cessation of crop growth. As noted by the Panel in response to previous questions in this report, the laboratory studies utilizing soils or soil microcosms provide interesting leads as to what might happen in the field. However their relevance to field conditions remains unknown.

In attempting to understand the results described above, several Panel members noted that Cry proteins are essentially biochemically similar to other proteins, and the fate and transport of Cry proteins can be expected to be similar to that of other proteinaceous materials entering soil. Protein entering the soil ecosystem is available for microbial degradation by heterotrophic organisms, including bacteria, and actinomycetes and fungi, under aerobic or anaerobic soil conditions. However, proteins have been shown to be adsorbed to colloidal materials including clays and humus, which protects the proteins against biodegradation (Stotzky, 2000). Such adsorption is likely to increase in soils high in clay or organic matter content. One Panel member added that although it has been shown that sorbed proteins and DNA can remain active, it needs to be demonstrated that these sorbed materials are biologically active. Indeed, there may be a correlation between biological activity and bioavailability for degradation. Under field conditions, the soils in which cotton and corn are grown typically have pH values between 6 and 8, a range in which soil microbial degradation of Cry toxin is usually rapid. In general, studies have shown that

initial degradation of Cry protein is rapidly followed by reduced rates of degradation, perhaps due to soil sorption and therefore reduced bioavailability. Relatively few researchers have demonstrated that Cry toxins that persist in soils remain biologically active. Tapp and Stotzky (1998) used toxicity assays with a susceptible insect species to show that Cry toxins persist in non-sterile soils for more than eight months. Thus, in this case, which is only case involving a transgenic crop where data are available, biological activity correlates positively with persistence. However, there is no evidence that persistence has adverse environmental effects.

Potential already exists for obtaining needed data from fields where Bt crops are currently planted. Ideally, however, it would be desirable to begin new studies in fields not planted previously with Bt crops, and follow these fields for several years. Further it would be desirable to document environmental conditions in the field and analyze key soil biota to assess possible changes with the introduction of Bt crops. This would allow the gathering of baseline data before experiments on accumulation are begun. Overall, the Panel agreed there is a lack of data on the influence of Cry proteins on soil microbial populations. Research is also needed to determine what kinds of changes in the soil microbiota are considered a hazard, and what levels of change are acceptable for risk assessment. A great deal of variation already occurs between crops, fields, and seasons. Thus, while the Panel was in agreement that these studies should be pursued, the consensus was that the Agency should be able to complete its preliminary risk assessment in the absence of such studies.

With respect to the soil microorganism studies, one Panel member thought it important to investigate the effects of Cry proteins on the microbial community, because microorganisms are primarily responsible for biogeochemical cycling in soil, which is necessary for sustained crop production and soil quality. Such studies should include the effects of Cry proteins on the activities of microorganisms in soil (e.g., mineralization of carbon, nitrogen, and other essential elements; fixation of dinitrogen; degradation of xenobiotics and other pollutants; grazing of protozoa and nematodes on the microflora). These studies should emphasize investigation of the population dynamics of the soil microbiota using techniques of molecular biology (e.g., single-strand conformation polymorphism, denaturing and temperature gradient gel electrophoresis) to determine the effects of the Cry proteins on species diversity. For risk assessment, this Panel member considered studies of 30 days duration with worst case scenarios, using maximum Cry protein concentrations, to be appropriate. Another Panel member did not think such studies were necessary to assess risk because Cry proteins are toxic to invertebrates, not microorganisms as far as is known, and because these types of studies are not required for chemical insecticides.

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?

The environmental fate data and horizontal gene transfer assessment appear to be

adequate, and no additional data are probably necessary for the Agency to complete a risk assessment. Horizontal gene transfer from plants to microbes has not been demonstrated in non-sterile soil, nor is there a well-defined mechanism for such transfer. Even bacteria-to-bacteria transfer frequencies in non-sterile soil are extremely low, particularly if there is no selection pressure to drive such transfer. Such bacterial transfer can be by conjugation, transformation, or transduction. Transfer from plant to bacterium would most likely occur via transformation, if Bt DNA within crop residues survived long enough to allow the transfer process to occur. Reported transfer frequencies in the literature for transformations are low. However, DNA from various sources binds rapidly on clays and humic substances, the bound DNA becomes resistant to biodegradation, and the bound DNA retains the ability to transform competent bacterial host cells. As an example, Stotzky (2000) indicated that bacterial DNA could persist in soil. If the bound DNA encounters transformable bacteria, it may transform them. The potential hazards of such gene transfer are not clear, and the ability of a bacterium receiving *cry* genes to compete in the soil environment might be low, as a result of having to expend energy for the synthesis of Cry proteins. Nevertheless, because of the general concern about the environmental consequences of horizontal gene transfer, the possibility of the transfer of transgenes from plants to microorganisms in soil warrants further study.

Whether or not transfer could take place, consideration should be taken into account that Bt is a soil-borne bacterium prevalent in many soils. Thus, Bt genes are already naturally present in many soils, and yet no demonstrated horizontal gene transfer of such genes has been observed. One of several possible explanations is that such transfer provides no selective advantage for the potential recipients. Another explanation is that such transfer has been insufficiently investigated. As stated previously, the fate of Bt genes is either rapid degradation or sorption to colloidal particles.

REFERENCES

Crecchio, C. and G. Stotzky. 1998. Insecticidal activity and biodegration of the toxin from Bacillus thuringiensis subsp. kurstaki bound to humic acids from soil. *Soil Biol. Biochem*. 30, 463-470.

Palm, C. J., D. L. Schaller, K. K. Donegan, and R. J. Seidler. 1996. Persistence in soil of transgenic plant produced Bacillus thuringiensis var. kurstaki d-endotoxin. *Can. J. Microbiol*. 42, 1258-1262.

Pruett, C. J. H., H. D. Burges, and C. H. Wyborn. 1980. Effect of exposure to soil on potency and spore viability of Bacillus thuringiensis. *J. Invertebr. Pathol*. 35, 168-174.

Saxena, D. and G. Stotzky. 2000. Insecticidal toxin from Bacillus thuringeniensis is released from roots of transgenic Bt corn in vitro and in situ. *FEMS Microbiol. Ecol. 33, 35-39.*

Saxena, D. and G. Stotzky. 2001. Bacillus thuringiensis (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and

fungi in soil. *Soil Biol. Biochem., (in press).*

Saxena, D., S. Elores, and G. Stotzky. 1999. Insecticidal toxin in root exudates from Bt corn. *Nature 402:480*.

Sims, S. and J. E. Ream. 1997. Soil inactivation of the Bacillus thuringiensis subsp. kurstaki Cry11A insecticidal protein within transgenic cotton tissue: Laboratory microcosm and field studies. *J. Agricul. Food Chem.* 45, 1502-1505.

Stotzky, G. 2000. Persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis* and of bacterial DNA bound on clays and humic acids. *Journal of Environmental Quality*. 29:691-705.

Tapp, H. and G. Stotzky. 1995. Insecticidal activity of the toxins from Bacillus thuringiensis subspecies kurstaki and tenebrionis adsorbed and bound on pure and soil clays. *Appl. Environ. Microbiol*. 61, 1786-1790.

Tapp, H., and G. Stotzky. 1998. Persistence of the insecticidal toxin from Bacillus thuringiensis subsp. kurstaki in soil. *Soil Biol. Biochem.* 30, 471-476.

Tapp, H., L. Calamia, and G. Stotzky. 1994. Adsorption and binding of the insecticidal proteins from Bacillus thuringiensis subsp. kurstaki and subsp. tenebrionis on clay minerals. *Soil Biol. Biochem. 26: 663-679.*

West, A. W., and D. Burges. 1985. Persistence of Bacillus thuringiensis and Bacillus cereus in soil supplemented with grass or manure. *Plant and Soil.* 83, 389-398.

West, A. W., N. E. Crook, and H. D. Burges. 1984. Detection of Bacillus thuringiensis in soil by immunoflurescence. *J. Invertebr. Pathol*. 43, 150-155.

Non-target Organism Effects Assessment Questions

1. The Panel is requested to provide comments on the Agency's weight of evidence assessment and its conclusions 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.

The Agency has reported results from Tier 1 laboratory tests where high doses of Bt Cry1Ab protein (hazard dose at 10-100 X effective environmental concentration) have been fed to various animal species. The Panel agreed that the results from these tests suggest that Cry1Ab proteins are unlikely to have significant adverse ecological effects on populations of wild mammals, birds, non-arthropodan invertebrates, and aquatic species. Small-scale field trials also indicate that (excluding insect relatives of target species) adverse ecological effects on other

Arthropoda are likely to be small, if they occur at all.

One Panel member recommended that tests for the effects of pollen from transgenic plants on Coccinellids (lady beetles) should use species that tend to feed on pollen more than *Hippodamia convergens* which feed on pollen rarely if, at all, in the field. It should be considered that perhaps *Coleomegilla maculata* would be a more appropriate test species since it is more of a generalist feeder than *H. convergens*. In addition, *C. maculata* is readily available commercially, making it a practical test species. The same Panel member suggested the Agency should obtain information on Bt expression levels in potato and cotton pollen, as well as in corn. Such data would provide more information about the exposure level for pollinators of these crops, and provide further insights for the Agency.

Since the Agency is required to consider potential for negative impacts in comparison to possible alternatives, it is important to note that the Agency's background document cites studies in which researchers found increases in species diversity in four Florida Bt sweet corn plots compared to non-Bt corn conventional control plots. Higher populations of beneficial and non-target insects were also found in the Bt sweet corn fields. These increases were most likely associated with the decreased use of broad-spectrum insecticides possible when Bt sweet corn was grown. This pattern needs to be demonstrated in Bt field corn and northern sweet corn fields before conclusions can be drawn regarding the ecological impacts of Bt corn on species diversity.

A reference was made to the tendency for some syrphid flies to accumulate pollen and the possible effects that Bt corn pollen might have on these flies. One Panel member noted that previous studies with natural Bt Cry1 proteins found no adverse effects on muscoid flies belonging to the dipteran suborder Cyclorrhapha, the same taxonomic suborder to which syrphid flies belong. Another Panel member concluded that because Bt corn provides new routes of exposure and the protein delivered to the syrphid is not identical to that in the bacterium, there is a possibility that the effects of Bt corn pollen may be different from the effects of natural Bt toxins.

Even though the effects of transgenic Bt cotton and corn can reasonably be expected to be minor, a few Panel members thought that the Hilbeck data relating to effects of Bt toxin on lacewings should not be dismissed by the Agency in their risk assessment. The Hilbeck data was dismissed by the Agency based on standards that were not applied to all of the work reviewed by the Agency, and the Hilbeck work was singled out for an excessively critical analysis. Control mortalities were not unusually high (especially compared to control mortalities in other studies reviewed favorably by the Agency), dead insects were not fed to *Chrysopa*, the no-choice experiments were designed to detect potential hazard (just like the earthworm, collembola, and *Daphnia* trials), the observed effect was not small (30% increase in total immature mortality), and the Agency should have concluded that a potential hazard to *Chrysopa* had been identified. However, another Panel member pointed out that in published field studies carried out in Bt corn, no adverse effects on *Chrysopa* populations were found.

In addition, other Panel members commented that the December, 1999 SAP suggested "Most scenarios for secondary exposure are not very compelling because of the low concentration of active ingredient likely to be delivered to non-target insects or the small fraction of the non-target insect population likely to be affected. The half-life of a pesticidal protein also is expected to be short. Therefore, the Panel suggests that food chain effects are not likely to be significant."

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.

The Agency should be commended for responding rapidly to the monarch issue and its involvement with the consortium of scientists that are conducting research in this area. The Agency's participation in the USDA–sponsored workshop in February, 2000 that presented results from the 1999 field season and identified research priorities regarding Bt corn and monarch butterflies for the 2000 field season was indicative of its high level of interest.

The information presented at the February, 2000 workshop and a previous monarch meeting (Chicago, November, 1999) provided the basis for the Agency's preliminary analyses. The Panel was divided concerning the Agency's interpretation of this information. Some Panel members thought that the Agency was overly optimistic when it determined that Bt pollen posed little to no risk to monarchs. Other Panel members thought that the Agency's interpretation of existing data was appropriate. All Panel members did agree, however, that the final assessment must incorporate information that will be reported at the November, 2000 Bt corn/monarch workshop. The Panel sees the results of field tests that directly assess possible impacts of Bt pollen on monarch larvae as particularly important. This is addressed in more detail in response to question 4.

Several Panel members noted that the Agency overlooked several published sources of literature relevant to the analysis. For example, the literature in weed science about the establishment and population dynamics of milkweed in agricultural habitats with and without herbicide applications should be considered. In addition, recent monarch ecology research was not included in the analysis.

A few Panel members believed that the Agency must consider several studies that had not been made publicly available before finishing a final analysis. They were aware of the results from three of these field studies, which were conducted during pollen shed in Ontario, Canada; Maryland, and Iowa. These studies suggested to them that Bt corn (specifically events MON810 and Bt 11) did not impact monarch larval development relative to non-Bt corn. These Panel members concluded that because no mortality of monarch larvae exposed to Bt11 and MON 810 corn hybrid pollen was observed compared with larvae exposed to pollen from hybrid isolines or non-Bt expressing hybrids, risk to monarch populations is minimal. The Panel acknowledged that this new information was not available to the Panel at the meeting. Thus, these conclusions reflect individual remarks and were not deliberated by the Panel.

The Agency also needs to take into account research from Minnesota, Iowa, Maryland and Ontario, Canada on the phenology of monarchs in cornfields and other habitats throughout the summer of 2000. These studies found that monarchs use cornfields as oviposition sites throughout the breeding season, even when corn plants are taller than milkweed plants. In the Iowa and Minnesota studies, per plant densities of monarchs were higher in cornfields than in non-agricultural habitats, but this was not found to be so in Ontario and Maryland. This research also showed that peak monarch abundance during the final generation before migration does overlap with pollen shed in Minnesota and Iowa. Peak monarch abundance during the final generation overlapped with pollen shed in Ontario and Minnesota, and occurred slightly after pollen shed in Maryland and Iowa. There was at least some overlap in all locations.

The Panel was divided over the Agency's conclusion that the Jesse and Obrycki (2000) lab study studying the effects on Bt corn pollen on monarch butterflies was not useful for risk assessment. One Panel member commented that E176 did cause mortality when milkweed leaves with naturally-deposited pollen were fed to larvae. Bt11 pollen only caused more mortality than control pollen when it was manually-applied to the leaves, and this effect may be due to anther contamination. While the Agency's assessment that additional field studies are important, lab studies may also be important to consider. However, other Panel members were critical of the Jesse and Obrycki (2000) study, highlighting several deficiencies in the research. This included: pollen collection and application procedures were not appropriate and could have resulted in contamination or improper dose, low numbers of larvae observed in trials with no replication of observations provided, and greater mortality of larvae exposed to a low dose of pollen, while larvae exposed to a higher dose had no mortality compared with the control group.

One Panel member agreed with the Agency's preliminary conclusion that mitigation can be achieved through development of hybrids that do not express Bt toxin in pollen. Another Panel member commented that developing Bt corn pollen that does not contain toxins could create a difficult tradeoff. Toxin-free pollen would avoid possible non-target risks, but it could result in more rapid evolution of resistance to Bt corn by target organisms. Second-generation neonate European corn borer can survive through the early instars by feeding on corn pollen rather than on leaves. With toxin-free pollen, larva might avoid toxin during the early, sensitive larval stages, only encountering toxin during the later stages, when they are much more tolerant of the toxin. This could result in more rapid resistance evolution.

There could be a beneficial tradeoff of using Bt crops compared to growing conventional crops due to an overall reduction in the use of synthetic chemical insecticides. This could benefit non-target organisms, including both beneficial and endangered species. The magnitude of this tradeoff will depend on the extent to which conventional chemical insecticides are used in non-Bt fields, and is expected to be higher in sweet corn fields and in the southern part of the corn belt. The Panel also had general comments about the Agency's background document which are provided below.

The Agency's background document indicates that the weediness of milkweed means that

it is subject to control measures and should thus be relatively rare in cornfields. In actuality, however, milkweed's resistance to weed control efforts makes it relatively common in cornfields. Research in Minnesota during the 2000 growing season suggests that cornfields are good milkweed and monarch habitats, and that more aggressive weed-control practices could have detrimental effects on monarchs. The level of weed control practiced by farmers is more likely to be a function of cost versus yield improvement rather than governed by indirect ecological benefits to nonrevenue producing species.

The Agency's background document suggests that harmful effects of Bt pollen will be mitigated by the fact that the pollen's biological activity is significantly reduced within approximately one week. However, exposure to toxic pollen over much less than this amount of time can be lethal. If pollen is shed over a period of two weeks in the field, some larvae could conceivably be exposed for their entire larval development period. What seems not to be well understood is how fast the toxin degrades in pollen after pollen shed.

The Agency's background document suggests that monarch exposure to toxic pollen will be limited because females prefer not to oviposit on milkweed surrounded by corn, or on leaves covered by lethal amounts of pollen. The summer 2000 phenology studies refute this position that females will not oviposit in cornfields. Female avoidance of plants covered with pollen may not reduce exposure significantly, since the larvae are the susceptible stage.

The Agency's background document suggests that larval feeding behavior will limit exposure, since neonates eat from the bottom of the leaf. However, late first instar larvae do chew through the leaf, as do all older stages. The fact that they eat from the bottom of the leaf may limit their ability to detect and avoid the toxin, but not prevent them from consuming it.

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

Generalizations are difficult to make, especially with respect to endangered species. Each species must be evaluated on a case-by-case basis. An evaluation of the Karner blue butterfly life history, habitat and distribution information suggest that Bt corn might have no or little impact on these butterflies. Two Panel members indicated that pollen distribution and dose-response studies have been conducted with the monarch butterfly suggesting that, unless Karner blue butterflies develop in cornfields, exposure to hazardous levels of Bt pollen might be unlikely. Two Panel members described circumstances where the Karner blue butterfly may come in contact with small amounts of Bt corn pollen. For example, wildlife managers may use Bt corn in wildlife management areas to augment food for wildlife. In addition, in some years, Karner blue butterfly phenology may be delayed sufficiently to overlap temporally with corn pollen shed. These conditions will be highly site specific. One Panel member suggested since Karner blue butterfly is an endangered species, a consultation with the US Fish and Wildlife Service (USFWS) as specified in the Endangered Species Act (i.e. Section 7 consultation) might be useful since the

USFWS is very familiar with the complex distribution of this butterfly. Other Panel members thought an informal consultation with USFWS may be useful, but that a Section 7 consultation was unnecessary.

4. Please comment on additional studies that 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.

The Panel agreed that field studies were the most direct way to assess non-target impacts. Studies in transgenic and near isoline non-transgenic fields allow scientists to directly assess possible impacts of transgenics on non-target species and also to determine if important community-level interactions occur. The Panel recognizes that, in particular, community-level interactions cannot be easily measured in laboratory experiments, but additional studies can strengthen the database for characterizing non-target effects. Specifically, studies that strengthen hazard analysis and those that strengthen exposure analysis are needed to complete a total assessment.

With the large plantings of Bt corn and Bt cotton that are now common in many states, the effects of these crops on non-target organisms should now be tested directly in the field. Any laboratory experiments should be ancillary to direct experiments in the field. It is evident from discussions on preceding questions that much of the data to properly assess the risk of Bt corn pollen to non-target lepidoptera (monarch butterfly populations) will soon be in the published scientific literature. It is important that these studies provide the Agency appropriate data on both the toxicity of the toxin and the likelihood of exposure by the non-target insect. These studies should include field-conducted bioassays, potential routes of exposure of non-target insects to the Cry protein and spatial and phenological relationships between crop and non-target species in question. Sound scientific findings must drive decisions of the Agency.
Inappropriate conclusions drawn from an insufficient database are to be avoided in this process.

Below is a list of additional studies that individual Panel members suggested, in no particular order. The Panel did not have sufficient time to prioritize these studies, but, if needed, prioritization could occur at a future SAP meeting:

Assessing the total landmass with milkweed and monarchs. It will be especially important to determine relative monarch populations in different kinds of habitat, including agricultural and non-agricultural areas. This needs to be a large scale study that includes surveys of randomly chosen plots.

Assessing the percentage of milkweed in different habitats. This will require knowing milkweed density in different habitats, and should be done in conjunction with the survey described above.

Continued study of the risk of exposure to toxic levels of Bt pollen. It is important to conduct surveys of pollen shed under different weather regimes, generating frequency distributions of

pollen densities on milkweed plants throughout anthesis.

Assessing the relative contribution of different regions to the monarch population that migrates to Mexico. This study needs to be conducted over several years, since it is likely that regional variations in weather conditions will cause contributions to vary.

Modeling population-level impacts of monarch mortality at different times during the season and at different life cycle stages. The relative risk of Bt pollen to monarch larvae must take into account the fact that mortality from this source might occur after other mortality has occurred.

Measuring susceptibility of monarch larvae to Bt pollen. Future studies of toxicity should be carried out in both field and lab settings, and should not be limited to neonates.

Identifying other at-risk Lepidoptera. Existing database surveys and field surveys should be used to determine other Lepidoptera that may be exposed to corn pollen. In addition, LC50 values for several species should be determined.

Evaluate buffer areas to promote biodiversity. Studies should be conducted to determine the best ways to promote biodiversity in agricultural buffer areas and set aside lands.

Reduced insecticide studies surveys. While research has shown that fields with transgenic plants have greater or equal numbers of non-target insects than fields where synthetic insecticides have been employed, data are needed to verify that the transgenic plants are actually replacing insecticides. Otherwise, a more suitable control would be untreated fields. As a matter of fact, to provide the most accurate comparisons, an untreated control should always be included.

Characterize Bt expression in all plant parts. The Agency needs to require data about toxin concentrations in all parts of a transgenic plant since such information will have significant implications for analysis of IRM plans and non-target species effects.

SAP Report No. 2000-07c, March 12, 2001

REPORT:
FIFRA Scientific Advisory Panel Meeting,
October 18-20, 2000, held at the Marriott Crystal City Hotel,
Arlington, Virginia

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Bt Plant-Pesticides Risk and Benefit Assessments: Benefit and Economic Analysis, Product Characterization and Human Health Effects

Mr. Paul Lewis
Designated Federal Official
FIFRA Scientific Advisory Panel
Date:_____

Stephen Roberts, Ph.D.
FIFRA SAP Session Chair
FIFRA Scientific Advisory Panel
Date:_____

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
October 18-20, 2000

SESSION I - Bt Plant-Pesticides Risk and Benefit Assessments:
Benefit and Economic Analysis, Product Characterization and Human Health Effects

PARTICIPANTS

FIFRA SAP Session Chair
Stephen Roberts, Ph.D., University of Florida, Gainesville, FL

FQPA Science Review Board Members
Frederick H. Buttel, Ph.D., Department of Rural Sociology, University of Wisconsin, Madison, WI
Margriet Caswell, Ph.D., USDA Economic Research Service, Washington, DC
Brian Federici, Ph.D., University of California, Riverside, Department of Entomology and Graduate Programs in Genetics and Microbiology, Riverside, CA
Steven Gendel, Ph.D, National Center for Food Safety and Technology, US Food and Drug Administration, Summit-Argo, IL
Ricki Helm, Ph.D., Arkansas Children's Hospital, Little Rock, AR
Terrance M. Hurley, Ph.D., Department of Applied Economics, University of Minnesota, St. Paul, MN
Hubert P.J.M. Noteborn, Ph.D., RIKILT, Department of Food Safety and Health, The Netherlands
Richard Williams, Ph.D., US Food and Drug Administration, Washington, DC

Designated Federal Official
Mr. Paul Lewis, FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, Environmental Protection Agency, Washington, DC

PUBLIC COMMENTERS

Oral statements were made by:
Mr. Carl Casale, on behalf of Monsanto
Mr. Leonard Gianessi, on behalf of the National Center for Food and Agricultural Policy
Mr. Jeff Stein, on behalf of Novartis Seeds
Kent Lanclos, Ph.D., on behalf of the National Cotton Council
Jane Rissler, Ph.D., on behalf of the Union of Concerned Scientists
Gary Munkvold, Ph.D., on behalf of Iowa State University, Department of Plant Pathology
Shelby Fleisher, Ph.D., on behalf of Penn State University
Mr. Clyde Sharp, Arizona cotton grower
Barbara Henry, Ph.D., on behalf of Aventis Crop Science
Val Giddings, Ph.D., on behalf of the Biotechnology Industry Organization
Mr. Bill Freese, on behalf of Friends of the Earth
Galen Dively, Ph.D., on behalf of the University of Maryland
Greg Nuessly, Ph.D., on behalf of the University of Florida
Mr. Dee Vaughn, on behalf of the National Corn Growers Association
Michael Hansen, Ph.D. on behalf of Consumers Union
Marlin Rice, Ph.D. on behalf of Iowa State University, Department of Entomology
Jason Hlywka, Ph.D. on behalf of Cantox Health Sciences Institute
Anne Bridges, Ph.D., on behalf of the American Association of Cereal Chemists

Written statements were received from:
Agricultural Biotechnology Stewardship Technical Committee
Auburn University
Aventis CropScience
Council for Agricultural Science and Technology
Dow Agrosciences, Inc.
Monsanto
National Cotton Council
North Carolina State University
Novartis Seeds, Inc.
Pioneer Hi-Bred Inc.
Union of Concerned Scientists

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency pertaining to Bt Plant-Pesticides Risk and Benefits Assessment: Benefit and Economic Analysis, Product Characterization and Human Health Effects Insect Resistance Management. Advance notice of the meeting was published in the *Federal Register* on September 6, 2000. The review was conducted in an open Panel meeting held in Arlington, Virginia, on October 20, 2000.

The meeting was chaired by Stephen Roberts, Ph.D. Mr. Paul Lewis served as the Designated Federal Official. Mr. Edward Brandt (EPA, Office of Pesticide Programs) summarized the Agency's benefits and economic analysis. John Kough, Ph.D. (EPA, Office of Pesticide Programs) discussed product characterization and human health effects.

In preparing this report, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This report addresses the information provided and presented within the structure of the charge by the Agency.

CHARGE

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?

DETAILED RESPONSE TO THE CHARGE

The specific issues to be addressed by the Panel are keyed to the Agency's background document "Preliminary Risk and Benefit Assessment for Bt Plant-Pesticides", dated September 20, 2000, and are presented as follows:

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?

The Agency's presentation helped the Panel understand several parts of the Agency's analysis that were not clearly explained in the Agency's background document. The Agency's background document does not adequately explain the methods or the assumptions that are used in the analysis. Thus, it is difficult to evaluate the benefits and economic analysis assessment. A partial budgeting approach is used in a simulation of on-farm profitability from Bt technology adoption. Costs are not directly obtained, so there is no way to assess costs of refuge requirements or how other factors of production change with the use of the technology. The analysis only indirectly compares costs on actual Bt acreage. The benefits assessment appears to only evaluate whether farmers find the adoption of Bt crops to be profitable, and to what extent. Because Bt products are successfully marketed, we can presume that they are expected to be profitable by those who purchase the seed; that is, that private benefits (revenues) exceed private costs. This is an extremely narrow interpretation of the total benefits. As a public entity, the Agency should ensure that net social benefits are positive, where the social accounting includes private, environmental, consumer, and other benefits and costs. The Panel's concern about the scope of the benefits analysis is addressed in the response to question 4. The following discussion in response to question 1 only relates to the assessment of farm-level profitability.

The Panel expressed a concern about the use of uniform distributions to characterize both the benefits and costs of farm production with Bt crops. A sensitivity analysis was conducted, but it is not clear how the methodology specifically was implemented to compare the uniform and normal distributions. The explanation of the sensitivity analysis was insufficient to interpret the results. Data suggest that there is a wide distribution of profitability performance associated with the use of Bt corn. For example, data for Wisconsin collected by the University of Wisconsin Program on Agricultural Technology Studies have revealed that 46.3 percent of 1999 Bt corn users reported that net profits were the same as or lower than with non-Bt corn when compared with the relevant conventional variety. The Wisconsin data also show that the profitability and overall performance of Bt corn varieties in Year 1 is positively associated with use of Bt corn in

year 2, and that factors other than profitability (e.g., perceived marketing uncertainty) affect adoption. These data do not support the notion that in a given year, adoption of Bt corn is a function simply of seed cost and yield response.

The Panel offered several suggestions to improve the analysis based on the assumption that there is a distribution of expected profitability among farmers based on an underlying distribution of characteristics (economic conditions, policies, human capital, natural resource assets, weather, pest pressures, etc.). No intuitive reason was given why this distribution would be uniform. With respect to the distribution of net benefits, the Panel suggested:

1. Use a single distribution for net benefits rather than separate distributions for benefits and costs.

2. Calculate unknown parameters directly if possible. This may be easier than the numerical method that was used.

3. Explore different distributions. The normal distribution is probably the best place to start. With the normal distribution, there is a need to provide a reasonable estimate of the mean or variance of the net benefits. The actual adoption rate can be used to set the remaining parameter since the analysis is a simulation rather than an optimization. This methodology can be used to produce a demand curve for calculating the producer surplus from purchasing Bt corn, which provides an estimate of the benefit of adoption to growers.

4. The analysis would be strengthened by including a sensitivity analysis on several of the variables such as crop price, seed cost, adoption levels, and yield effect. There is a likely distribution for these factors that currently are considered as point estimates. Seed premiums for Bt corn have been declining since 1997 due to industry competition, which should be included in the analysis. The effect of short- and long-term impacts on export sales (and hence on net income and returns) due to consumer resistance to plant-pesticides, and to regulatory decisions in other countries can be included within the sensitivity analysis framework.

Benefit and cost calculations should consider the declining adoption of Bt corn (as well as rising adoption of other Bt crops) in the 2000 growing season, and how lower adoption of Bt corn has affected the estimation of benefits and costs. There is evidence from the data collected by the Program on Agricultural Technology Studies at the University of Wisconsin, Madison that Bt corn adopters in a given year have a substantial probability of discontinuing use in a subsequent year. The Wisconsin data show that 40 percent of Bt corn adopters in 1998 had discontinued Bt corn use by 1999, and 25 percent of 1999 Bt corn users had continued Bt corn use by 2000. Wisconsin data, of course, should not be generalized to the U.S. because of the low ECB pressure in Wisconsin and the fact that Wisconsin is a peripheral Corn Belt state.

The methods and analysis for estimating grower demand for Bt cotton in Hubbell, Marra, and Carlson (2000) provided a rigorous statistical approach that might be effectively used in the

Agency's analysis. A drawback with the approach is that it cannot be used with data regularly generated by the USDA or others. The Fernandez-Cornejo and McBride (2000) methodology using the USDA/NASS Agricultural Resource Management Study (ARMS) also provides a statistically rigorous estimate of demand.

Comparing the Hubbell, Marra, and Carlson analysis to the Fernandez-Cornejo and McBride study, the former approach will tend to capture more of the intangible benefits and costs to growers of using Bt plant-pesticides and will provide a more accurate assessment of demand. It will also capture the expected value of Bt plant-pesticides over time and not the actual value for the given year. The EPA methodology is similar to Hubbell, Marra, and Carlson in this regard. The primary benefit of the Fernandez-Cornejo and McBride approach is that it uses data that are more readily available. The Hubbell, Marra, and Carlson analysis provides a better consistency check for the EPA methodology because the unit of measure is similar. However, care must be taken in making this comparison since the survey on which the analysis is based was limited in scope. Comparisons with Fernandez-Cornejo and McBride should only be made if the results are averaged over several years.

The profitability of adopting Bt plant-pesticides will vary substantially across geographic locations. For instance, data suggests that Wisconsin does not suffer from as frequent or severe ECB infestations as Minnesota and Illinois. Given that production regions are heterogeneous, estimates of grower benefits would be improved by breaking up the analysis into more homogeneous regions. Regions could be defined based on type and severity of the pest complex, average yields, and marketing regions. Regions with similar pest complexes and average yields will experience similar yield losses and cost savings due to reduced pesticide use. Marketing regions are important especially since there is resistance to commodities produced with Bt plant-pesticides in some markets. For example, growers who produce primarily livestock feed may tend to value Bt corn more than growers producing for export.

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.

The Agency's background document did not give adequate information for the reader to ascertain what USDA National Agricultural Statistics Service (NASS) data were used and why. Other sources of information also were used, and it was not clear why they were chosen when NASS figures were available. There may be good reasons, but the reader was left with the impression that some sources were used due to their magnitude (and thus predetermine the result). For example, EPA used a 175 bu/acre figure in the model instead of the 134 bu/acre USDA figure for 1999 corn yield. The difference between the two numbers greatly affects the calculation of benefits. In another case, EPA used an average per acre net benefits figure of $11 for cotton when at least eight other studies reported a value of around $40 per acre.

There is a need for the use of systematic farm-level data to estimate benefits. Farm-level

data, however, must be collected carefully in order to assist in making reliable estimates about benefits and costs. Survey data need to be collected through random sampling, involve data-sets with high response rates of 50 percent or more, involve a neutral entity administering surveys, and have neutrally posed questions. NASS surveys often are the best source of these data. NASS data do not cover all crops and regions of interest to the Agency, however, which has led to extrapolations that are questionable. For example, for the adoption of Bt sweet corn, the Agency background document (p. 10) noted that the average figure for Bt vegetables taken from the NASS Pest Management Practices report. There was no explanation of why the level of adoption for sweet corn would be identical to that for all vegetables or why that particular survey was used. On p.4, the Agency gave their own estimates of corn adoption which is close to USDA figures, but not quite the same. There was no explanation of how the estimates were derived or why they differ from the USDA estimates.

The comparisons of means, especially for aggregated data, can be very misleading. This point is acknowledged on p. 13, but is seemingly ignored throughout the rest of the analysis. Even if one was able to associate actual yields and pesticide use with those who adopt and those that do not, comparisons of means is difficult due to selection bias. To take average adoption rates (or market shares from the hypothetical distribution) and compare with state average pesticide use for the crop is a questionable approach.

The Agency may be able to make further use of NASS chemical and farm input use surveys. The Panel recognized the limitations of NASS surveys with respect to the coverage of crops and regions, but it may be possible, at least in the case of cotton, to construct annual datasets for counties on Bt variety use, yields, use of various chemicals, and target pests. One could then examine the correlations and regression slopes for the impacts of Bt cotton use over time, and also include control variables in the models. The analyses could also be disaggregated by region, with regions being chosen in a more fine-grained way than by dichotomizing states into high-adoption and low-adoption states.

The Fernandez-Cornejo and McBride (2000) study provides a useful approach for using the NASS Agricultural Resource Management Study survey data to assess whether Bt plant-pesticides have actually decreased pesticide use. The methodology also supports rigorous statistical testing for significant differences, which is not currently the case for the EPA methodology. A less rigorous non-parametric test may be adequate for establishing the statistical significance of differences in Bt plant-pesticide adoption rates and pesticide use.

The use of parameters from case studies can bias the analysis. For example, the use of elasticities from a narrow analysis for extrapolation to a national level will determine the magnitude of changes in profits from adoption. If the Agency wants to foster transparency, terms like “elasticity” must be defined in a way that could be understood by a non-economist. The Agency used the elasticities reported in the Fernandez-Cornejo and McBride (2000) study of the cotton crop budget of Mississippi to extrapolate the increase in variable profits to the national level (p. 19). This extrapolation is highly questionable because the elasticities, strictly speaking,

apply only to the Southeast region. Elasticity estimates for other regions need to be obtained to ascertain whether the Mississippi figures represent national conditions. At the minimum, a sensitivity analysis should be conducted to see how the results would be changed if elasticities varied.

The analysis incorrectly interprets the Hubbell, Marra, and Carlson survey study of Bt cotton (p. 19). Their estimate of net benefits (including insecticide use changes, Bt seed premium, and yield changes) is based on same-farm comparisons, which reduces selectivity bias. The estimate of benefits for the selected states and for the study year should not be extrapolated to the entire cotton belt or for other years.

The Agency's background document does not include adequate data on compliance with refuges and resistance management. Sound farm-level survey data are essential for addressing this issue. It is critical to collect farm-level data in areas with very high use of Bt varieties since such information is needed to assess the impact of refuges and required practices on profitability.

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

The Agency may be able to make further use of NASS chemical and farm input use surveys. The Panel recognized the limitations of NASS surveys with respect to the coverage of crops and regions, but it may be possible, at least in the case of cotton, to construct annual datasets for counties on Bt variety use, yields, use of various chemicals, and target pests. One could then examine the correlations and regression slopes for the impacts of Bt cotton use over time, and also include control variables in the models. The analyses could also be disaggregated by region, with regions being chosen in a more fine-grained way than by dichotomizing states into high-adoption and low-adoption states.

The Fernandez-Cornejo and McBride (2000) study provides a useful approach for using the NASS Agricultural Resource Management Study survey data to assess whether Bt plant-pesticides have actually decreased pesticide use. The methodology also supports rigorous statistical testing for significant differences, which is not currently the case for the EPA methodology. A less rigorous non-parametric test may be adequate for establishing the statistical significance of differences in Bt plant-pesticide adoption rates and pesticide use.

It is not surprising that there have been reductions in use of ECB, bollworm, and budworm pesticides as a result of the use of Bt varieties. The evidence presented in the Agency's background document, however, has significant shortcomings in measuring the reduction. For example, one concern is that the data show no reduction in the use of ECB insecticides in the low-adoption states associated with use of Bt corn varieties. The major data need is for scientifically sound primary farm-level data collection (involving representative sampling, high response rates, and neutral administration and question wording). Both farm-level and aggregate-level data are needed. Farm-level data are needed to understand the degree to which

pesticide use reduction opportunities are actually taken advantage of, and why not (e.g., risk aversion, lack of information). Aggregate data and farm-level data can be mutually validating.

There is a need to systematically take into account other factors (increases/decreases in public investments in IPM, changes in insect pressures) that shape use levels of Bt crops and use levels of particular pesticides.

The analyses of the chemical use implications of Bt crops should consider the particular case of "stacked" (Bt and herbicide resistant) varieties, since the balance of increased/decreased chemical use is likely to be quite different than the case of Bt-only varieties.

Establishing a link between changes in pesticide use and environmental and health benefits is difficult. Showing a statistically significant decrease in the use of more hazardous pesticides is a good starting point, but if the Agency wants a more quantitative measure, it needs to calculate changes in exposure of humans, wildlife, and beneficial insects and to value those changes in exposure. The Agency can use models of the transport and fate of pesticides in the environment in order to estimate changes in exposure if the analysis is expanded to include non-farm benefits of Bt crop use. This is described in more detail in response to question 4.

To value changes in exposure, one can look at expected changes in mortality and morbidity. For humans, there are a wealth of estimates of the value on increased mortality and morbidity. For wildlife, there are data in the contingent valuation literature.

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?

The Panel expressed a broad concern that the level of analysis of the EPA's benefit estimates is not in balance with the risk assessment. The introduction of Bt plant-pesticides is perceived to have many potential risks and many potential benefits. These include both direct and indirect effects. The EPA analysis includes the investigation of a wide array of both direct and indirect risks, but the analysis does not cover the full range of direct benefits nor any indirect benefits. Certainly, if the weight of evidence is in favor of the benefits relative to the risks, then there is no need to enumerate more benefits. However, if this is not the case, a more comprehensive benefit assessment is necessary.

The only benefits that are assessed are those that are represented by farm-level profits of Bt adopters. This is a very narrow scope for the analysis. Private monetary profits gained by a single sector are compared with all the public and private risks that are potentially associated with Bt adoption. If there are public benefits or private benefits to other sectors to be gained from the use of the technology, those benefits should be included. The assessment does not deal with general equilibrium effects that might be expected with wide-scale adoption. These would include impacts on consumer prices, competitiveness, and industry structure. There has been a substantial

increase in corn yield due to the adoption of Bt corn (60 million bushels) and a subsequent decrease in damage due to ECB. The price of corn was affected by this supply increase as well as the expectations farmers have for potential yield. Other dynamic effects could be considered. For example, there is also the potential that widespread adoption of a single crop variety will increase the vulnerability of commodity supply to a pest infestation.

The benefits assessment document clearly states that the analysis does not include changes in commodity prices, shifts in benefits among producers and consumers, impacts on foreign trade, registrant profitability, changes in environmental quality, changes in farm worker and food safety (e.g., reduced levels of mycotoxins), and incentives for product development. Environmental benefits from potential reductions in pesticide-induced damage to beneficial insects or wildlife also are not considered in the analysis. Many of these benefits may be small and/or hard to estimate, however, others are not. While changes in commodity prices, foreign trade, and incentives for product development represent indirect benefits, registrant profitability is direct. At the very least, all direct benefits should be included in the assessment. There are other production costs that may be associated with the adoption of Bt crops. For example, planting Bt may be easier to do than following a complex IPM plan or using certain chemicals. Other practices may be changed which will change costs of production.

Estimates suggest that the benefits to registrants can actually be higher than the benefits to growers. Investments in research and development that brought Bt plant-pesticides to market are sunk costs. These costs do not change regardless of whether the registrant produces one or one million bags of seed. The additional amount that the registrant sells the transgenic seed for is a rough estimate of registrant profitability (note the estimate will be upward biased, but will be much more reasonable than zero). The seed premium is the return on the company's R&D investments, and it provides the incentive to innovate.

A more comprehensive analysis considering direct and indirect benefits (consumer, producer, foreign trade, and registrant benefits) could follow along the line of Falck-Zepeda, Traxler, and Nelson (2000) and Moschini, Lapan, and Sobolevsky (2000). These analyses can probably be reconstructed for each crop and year using existing data. The most complete economic study of Bt cotton adoption data was reported in Frisvold, Tronstad, and Mortensen (2000). The study was based on entomologists' estimates of yield and insecticide cost changes, industry estimates of Bt cotton acreage, and the best available estimates of cotton demand and supply conditions. They included value to farmers, both domestic and foreign cotton consumers, and biotechnology and seed companies.

The Agency could pay more attention to the substitution of pesticides in corn and changes in pest complexes. The results of the Agency's analysis for corn suggests that pesticide use for chemicals targeting the ECB have fallen, but pesticide use targeting other pests has increased. If ECB control provided by Bt corn results in a new economically significant pest, then the benefits of Bt corn in terms of reduced pesticide use will not be as large. Resistance can also impact the value of Bt corn, but Integrated Resistance Management plans are designed to possibly prolong

the value of all effective technologies.

References

Fernandez-Cornejo, J., and W. McBride. 2000. Genetically Engineered Crops for Pest Management in U.S. Agriculture: Farm Level Effects, Resource Economics Division, Economic Research Service, U.S. Department of Agriculture, Agricultural Economics Report, No. 786.

Falck-Zepeda, J., G. Traxler, and Nelson. 2000. Surplus Distribution from the Introduction of Biotechnology Innovation. *American Journal of Agricultural Economics*, May, 2000.

Frisvold, G., Tronstad, and Mortensen. 2000. Adoption of Bt Cotton: Regional Differences in Producer Costs and Returns, Beltwide Cotton Conference, in press.

Hubbell, B., Marra, M., and G. Carlson. 2000. Estimating the Demand for a New Technology: Bt Cotton and Insecticide Policies. *American Journal of Agricultural Economics*. February, 2000.

Moschini, G., H. Lapan, and A. Sobolevsky. 2000. Roundup Ready Soybeans and Welfare Effects in the Soybeans Complex. *Agribusiness*, 16, pp. 33-55.

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?

The consensus of the Panel was that there were two concerns related to exposure/consumption of proteins - acute toxicity and allergenicity. The Panel believed that it is not possible to establish an exposure threshold for all proteins, but that well established protocols exist for testing individual proteins for acute toxicity. The Panel consensus was that it is not currently possible to identify conservative threshold levels for allergenicity.

The Panel supports evaluation of Bt-pesticidal proteins by acute dose-range animal studies and, if required in exceptional cases, by semi-chronic repeated exposure as has been outlined by the June, 2000 SAP. This testing should be done via a representative route of exposure (i.e. oral). For such studies, standard test protocol procedures should be applied according to existing guidelines. Histological observations on target organs and the digestive tract should supplement the toxicological data. Conventional approaches to toxicity testing on the basis of dose-response relationships may establish the dose-level of the Cry protein that does not represent toxicity and thus, should be assigned as the No Observable Adverse Effect Level (NOAEL) or threshold level. The plant-expressed Cry-proteins currently under consideration do not show an adverse response in maximum hazard dose studies. For these non-toxic Cry-proteins, a threshold level could be set in the range of the maximum dose tested. A more conservative approach would be to use the

maximum dose levels used in the 30-day sub-acute repeated dose study, which may still be in the order of 10,000 times or higher than the level produced in the Bt-crop plant.

The consensus of the Panel was that the issue of allergenicity is much more challenging. One member stated that from the perspective of IgE-mediated food allergy, there are two essential criteria that need to be addressed: allergen sensitization and the allergic response. The mechanism(s) of food protein allergic sensitization are still unknown and threshold limits cannot be determined. With the introduction of new food sources and the ready availability of novel foods to the consumers of the world, there are no criteria to establish a sensitization level of any particular food protein.

The mechanisms of allergic sensitization are complex and include: 1) genetic predisposition, which is multifactorial and includes several genes and gene families as well as known and unknown HLA allergen-associations; 2) characteristics of the protein and protein source; 3) environmental circumstances which include dietary exposure and time of entry into the diet, confounding factors such as tobacco smoke and diesel exhaust that may contribute to recognition of dietary and other proteins as allergens; 4) immunizations and infections (the "hygiene hypothesis"); and 5) other additional unknown factors that may lead to inflammatory responses altering the balance of Th1/Th2 cells that characterize an IgE-mediated disease. Potential routes of food exposure that can lead to production of specific IgE antibody include: 1) the placenta; 2) breast milk during lactation; 3) formula feeding; 4) solid food introduction into the diet; 5) accidental or covert introductions through food allergens as airborne droplets, floor dusts and by caretakers; and 6) organ transplants. Thus, there is no relevant criteria or data to suggest a threshold level for a food or food protein allergic sensitization.

With respect to sensitization, the amount and numbers of exposure of the antigen administered are the critical factors in the development of an immune response. Too little or too much may result in the development of tolerance (e.g. Atkinson et al., 1996). This means that we do not know the level of protein needed to sensitize a previously unexposed individual. This uncertainty highlights the importance of carrying out dose response relationships in model-systems. In addition, other constituents of the plant (so-called adjuvants) may influence the development of sensitization or affect immune modulation.

In regards to preexisting allergies, the Panel agreed again that it is not possible to establish a threshold. It is generally accepted that the human body is sensitized upon contact with the antigen, and that subsequent exposures provoke an allergic reaction. Upon subsequent exposure, the antigen will cross-link two surface-bound IgE molecules and trigger mast cells and basophils to release allergic mediators like histamines. Consequently, the tolerance level for food allergens could may be quite low, even one molecule would be theoretically sufficient (Peijnenburg et al., in press).

One Panel member suggested that the dose-level that does not show provocation, which means no experience of clinical symptoms in an allergic individual, also can be considered as the

threshold for elicitation. For example, in the case of peanuts, one of the most potent, perhaps the most potent, known human allergen, 100 micrograms has been proposed as a conservative elicitation threshold exposure limit (Moneret et al., 1998; Hourihane et al., 1997). In other experiments using oral challenges, 10 mg of lyophilized ovalbumin was required to induce symptoms in children (Lau et al., 1998), 6 mg of fish (Hansen TK, Bindslev-Jensen C. 1992), and 0.1 to 1 mg of peanut allergen (Hourihane JO, Kilburn SA, Nordlee JA, Hefle SL, Taylor SL). These data represent the beginnings of risk determinations for inducing allergic reactions, but do not address the issue of allergic sensitization.

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?

The consensus of the Panel was that the quality of the product characterization data presented was high, but that some additional factors should be considered by the Agency. A number of factors related to the genetic material and insertion event should be addressed. It appears that these factors are currently being considered in some detail, and in view of the fact the charge to the Panel related to the protein product was not the genetic event, these issues will not be considered further.

The Panel concluded that ideally the material used for testing should be produced in the appropriate plant system. However, the Panel recognized that low *in-situ* expression levels may not make this possible. The use of material produced in bacterial systems would be acceptable, assuming that potential differences between bacterial- and plant-produced materials are fully considered.

In addition to the test material itself, it is important to realize that the quality of any product characterization also depends on the nature of the reagents and probes used. The Agency should consider the reagents as well as the protocols used in evaluating the data presented. For example, antibodies are frequently used to characterize proteins in western blots or ELISA assays. It is important to use well characterized antibodies in these assays. Will the antibodies used detect peptide fragments or partial sequences as well as intact native molecules? If improperly folded or truncated molecules are present in the plants, will the antibody assays detect or measure them? This is important if ELISA assays are used to measure product concentrations in plant tissues. Do the glycosylation assays detect all modifications found in the plant species being considered?

It is important to demonstrate that protein production in an alternative host does not result in differences in post-translational modifications which alter behavior in subsequent experiments. Thus, the following considerations need to be addressed:

1. Identical behavior of the full length as well as the trypsinated forms of the protein on 2D-gel electrophoresis;
2. Identical immunoreactivity (i.e., binding) to poly- and/or monoclonal antibodies;

3. Identical patterns of post-translational modification (i.e., glycosylation) by using methods such as SDS-PAGE, that would allow the detection of the post-translational modifications in minor amounts of glycoprotein (alternative host versus crop plant);
4. It is not considered adequate to only sequence 10-15 N and/or C-terminal and up to three short internal protein fragments. Technology has advanced to assess larger segments, if not the whole protein; and
5. Toxicity to target insect species of the recombinant protein in comparison to the corresponding protein isolated from plants.

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 are necessary to assess the risk from the Bt-expressing plant-pesticide products?

The Panel believed that, although a great deal of relevant information is available, additional data needs should be considered by the Agency. Although the Panel agreed that the data submitted suggest limited evidence for a moderate risk to exposure of Bt plant-pesticide proteins, there is no criteria or data that would establish absolute allergenic risk assessments for this novel protein in the diet. Thus, the Panel had several suggestions regarding the collection and evaluation of human health-related data for Bt proteins.

In consideration of their structural composition, proteins should not be evaluated in the same manner as additives or chemical xenobiotics. The current testing regimes and data submitted to the Agency to support registration of the newly inserted Cry-pesticidal proteins (such as Cry1Ab) are sufficient to evaluate the inherent human toxicity of these Bt-toxins. More than 100 genes encoding Bt Cry proteins have been cloned and sequenced, and many of these vary in their insect target spectrum and toxicity to specific insects. This variation in specificity is attributed to the presence of different midgut binding sites in the insect. Several crops under development use combinations (pyramiding) of Bt Cry proteins as part of a resistance management strategy, and the safety of these crops will have to be evaluated on a case by case basis using existing EPA protocols.

Toxicity studies used in human health evaluations should comprise a combination of *in-vitro* and *in-vivo* animal experiments, and should be designed according to the nature and characteristics of the newly expressed substances in the plants. The rationale for this testing is the mode of action of the Bt-delta endotoxins such as Cry1Ab protein that have been studied extensively and elucidated (Sanders et al., 1998). Based on existing knowledge of Bt Cry protein mechanism of action, there is no evidence that these proteins will act similarly in mammals (Hoffmann et al., 1988, Noteborn et al., 1995).

The Panel supported the evaluation of Cry-pesticidal proteins by acute and, if needed subacute dose-range animal studies via a representative route of exposure in laboratory animals with new Bt-gene products for which no record of either toxicity testing or safe use is available. For such studies, standard test protocol procedures should be established by EPA and FDA and

applied accordingly. Animal studies with the whole trangenic plant are appropriate if there is reason to suppose that the introduced Cry-protein(s) may have altered the composition of the plant, i.e. in cases where trangenic plants are not substantially equivalent to their conventional counterparts. It should be realized, however, that animal feeding trials with whole food/feed products lack sensitivity and specificity, because it is physically impossible to administer such high doses of the candidate Bt crop through the animal diet and because confounding factors may influence the final result. Thus, as the Panel noted previously at the June, 2000 FIFRA SAP meeting, whole-feed testing with plant-pesticide containing food products does not provide a means to apply an appropriate margin of safety in studies directed toward the assessment of the plant pesticidal protein.

With regard to the Bt plant-pesticides under review, the Panel agrees with the Agency's viewpoint that when no effects are shown, even at relatively high dose levels in the acute oral exposure, the Bt plant-pesticides can be considered non-toxic. Therefore, genotoxicity studies including mutagenicity; chronic toxicity long-term administration; carcinogenicity long-term administration; reproductive toxicity studies during pregnancy; multigenerational studies etc. should not be required.

The Panel further suggests that the stability of introduced Bt-pesticidal gene products in the gastrointestinal tract should be tested by *in vitro* simulation of gastric and intestinal digestion and *in vivo*. Testing should be done with both the isolated protein and the same protein as part of a plant matrix because isolated proteins may be fully degradable in the simulated gastric system but survive gut passage intact when fed as part of a normal diet. If the new Bt protein plant-pesticide is stable under these conditions, testing should be extended to *in vivo* experiments. In cases where the Bt-gene product has been shown to be stable *in vivo*, bioavailability and transfer to body fluids (i.e. blood and milk) should be assayed, as well as potential allergenic effects.

With respect to allergenicity, the Panel concluded there is a continuing need to explore further approaches whereby the potency of allergic reactions of the isolated Cry-pesticidal protein and the transgenic plant can be more comprehensively assessed.

Two reports in the literature present information that will be valuable in the assessment of Bt proteins with respect to human health. In the first paper, Bernstein et al., (1999) conducted a health survey that included farm workers involved in the application of a Bt insecticide. These workers were surveyed before and after exposure to the Bt pesticide. Although the exposure route is primarily dermal or inhalation of complex Bt pesticide formulations that contained large amounts of spores in association with intact or partially assembled parasporal bodies containing toxin crystal, it does provide some information as to the potential allergenicity of the Bt proteins. The investigation results confirmed that both skin tests and antibody reactions were directed against the same Bt strain that was present in the commercial product used during spray operations. Intergroup comparisons between the prevalence of IgG and IgE immune responses indicated that exposure to Bt sprays could lead to allergic sensitization, as indexed by both positive skin tests and specific IgE and IgG antibodies. The results demonstrated that a

significant number of workers had IgG antibodies before the first spray, regarded as a reflection of previous exposures. In contrast, the increase in IgE antibody 1 month after spraying in one group of workers (designated high exposure workers) was regarded as consistent with an anamnestic response induced by exposure to classical allergens. Because the inhalation route sensitized only 2 of 123 workers and the symptoms were mild, the authors stated that it would be unlikely that consumers would develop allergic sensitivity after oral exposure to transgenic foods. Threshold levels were not evaluated. Only surveillance and clinical assessment of exposed individuals will confirm the allergenicity of Bt products or for any other novel protein introduced into the diet of consumers. The importance of this report is that reagents are available that could be used for reliable skin testing and serologic evaluation of Bt protein exposed individuals. Although this study does not address specific levels of Bt pesticides introduced into food sources and the resulting allergic sensitization and allergic response, significant factors remain, such as the protein level that will sensitize and evoke an allergic response and the acceptable prevalence rate for allergic responses to novel proteins. The results of this report clearly points out the need for surveys following exposure to pesticides as potential allergenic sources. The Panel recommends the Agency review this paper in its preparation of the risk assessment for Bt plant-pesticides.

In a second study, an investigation was undertaken to examine the systemic and mucosal adjuvanticity of Cry1Ac for the hepatitis B surface antigen and for bovine serum albumin in BALB/c mice (Vazquez et al,. 1999). The findings of this study suggest that Cry1Ac could be used as a convenient systemic and mucosal adjuvant carrier for protein antibody production. Although there was no assessment for IgE antibody, anti-HBsAg-antigen and anti-BSA serum antibodies of the IgM, IgG, and IgA isotypes increased significantly when both antigens were co-administered with Cry1Ac via the intragastric route, and the intestinal immune responses to HBsAg and BSA were enhanced when both antigens were co-administered with Cry1Ac via the intraperitoneal route. The authors found that the adjuvant potency of Cyr1Ac was similar to that of cholera toxin. Questions not addressed were the adjuvant effect of Cry1Ac co-administered with known allergens and animal and strain specificity as well as the adjuvant effect of seed storage proteins that humans consume routinely.

These two studies suggest that Bt proteins could act as antigenic and allergenic sources. Further, what is more relevant is that given appropriate conditions such as route of administration, selection of appropriate subjects, and experimental conditions, Bt proteins, and for that matter, any protein could potentially induce an immune response. What is not addressed, and should be, is the acceptable severity of the immune response as it relates to a significant health risk, the prevalence associated with the food source, the route of administration, and threshold levels for the protein to induce an immune response.

These studies, and future studies that suggest or investigate novel proteins as allergenic sources, should focus attention on novel proteins that produce true IgE-mediated reactions that manifest in a significant degree of severity in symptoms. Criteria should be established, based on available scientific evidence that addresses an acceptable level of symptomatic responses to novel protein. As with any other food source that enters the marketplace, knowledge of allergenicity is

revealed only after exposure to predisposed populations. While surveillance and reports from scientific investigators continue to reveal previously unidentified proteins as allergenic only after an allergic response is evident, there continues to be no known criteria for determining threshold levels of sensitization.

Finally, one Panel member noted that Bt was very common in the environment and that humans who eat fresh vegetables routinely consume and are exposed to thousands of Bt spores and Cry protein crystals daily.

REFERENCES

Atkinson, HAC, Johnson IT, Gee JM, Grigoriadou F, MillerK. 1996. Brown Norway Rat Model of Food Allergy. Effect of plant components on the development of oral sensitization. *Food Chem Tox*. 34:27-32.

Bernstein IL, Bernstein JA, Miller M. Tierzieva S, Bernstein DL, Lummun Z, Selgrade MK, Doerfler DL, Seligy VL. 1999. Immune responses in farm workers after exposure to Bacillus thuringiensis pesticides. *Environ Health Persp*.107:575-82.

Hansen TK, Bindslev-Jensen C. 1992. Codfish allergy in adults: identification and diagnosis. *Allergy*. 47:610-17.

Hofmann C, VanderBruggen H, Höfte H, van Rie J, Jansens S, van Mellaert H. 1988. *Proc Natl Acad Sci* USA 85:7844-7848.

Hourihane J O'B, Kilburn SA, Nordlee JA, Hefle SL, Taylor SL. An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut protein: A randomized, double-blind, placebo-controlled food challenge study. *J Allergy Clin Immunol* 1997;100:596-600.

Lau S, Thiemeirer M, Urbanek R, Kemeny M, Wahn U. 1998. Immediate hypersensitivity to ovalbumin in children with hen's egg white allergy. *Eur J Pediatr*. 147:606-8.

Moneret-Vautrin DA, Rance F, Kanny G, Olsewski A, Gueant JL, Dutau G., Guerin L. 1998. Food allergy to Peanuts in France -- evaluation of 142 observations. *Clin Exp Allergy* 28:1113-1119.

Noteborn HPJM, Bienenmann-Plourn ME, van den Berg JHU, Alink GM, Zolla L. Reynaerts A, Pensa M, Kuiper HA. 1995. *ACS Symposium* Series 605,134-137.

Peijnenburg AACM, Noteborn HPJM, Kuiper HA Biomarkers for evaluating the safety of genetically modified foods. In: Proceedings EMBODY 2000, Cambridge University Press. (in press).

Sanders et al. 1998. Safety assessment of insect-protected corn. In: Biotechnology and Safety Assessments 2nd. Ed (J.A. Thomas, ed).

Stone, TB; Sims, SR. 1993. Geographic susceptibility of Heliothis virescens and Helicoverpa zea (Lepidoptera: Noctuidae) to Bacillus thuringiensis. *J. Econ. Entomol*. 86, 989-994.

Vazquez RI, Moreno-Fierros L, Neri-Bazan L, de la Riva GA, Lopez-Revilla R. 1999. Bacillus thurigiensis Cry1Ac is a potent systemic and mucosal adjuvant. *Scan J Immunol*. 1999;49:578-84.