SAP Report No. 2000-06
December 1, 2000

REPORT

FIFRA Scientific Advisory Panel Meeting, November 28, 2000, held at the Holiday Inn Rosslyn Hotel

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

Assessment of Scientific Information Concerning StarLink™ Corn

NOTICE

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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.

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SAP Report No. 2000-06, December 1, 2000

REPORT:
FIFRA Scientific Advisory Panel Meeting,
November 28, 2000, held at the Holiday Inn Rosslyn Hotel,
Arlington, Virginia

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

Assessment of Scientific Information Concerning StarLink Corn

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
November 28, 2000

Assessment of Scientific Information Concerning StarLink™ Corn

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Designated Federal Official

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PUBLIC COMMENTERS

Oral statements were made by:

Rhona Applebaum, Ph.D., on behalf of the Alliance for Better Foods
I. Leonard Bernstein, Ph.D., on behalf of the University of Cincinnati
James BeMiller, Ph.D., on behalf of the Corn Refiners Association
Mr. William Bogot and Mr. Clint Krislov, on behalf of Krislov and Associates
Mr. Larry Bohlen, on behalf of Friends of the Earth
Anne Bridges, Ph.D., on behalf of the American Association of Cereal Chemists
Gary Burleson, Ph.D., on behalf of Burleson Research Technologies
Edmund Crouch, Ph.D., on behalf of Cambridge Environmental Inc.
Mr. W.J. Duensing, on behalf of the North American Millers Association
Mr. Bill Freese, a private citizen
Mr. Steven Gill, on behalf of the United States Department of Agriculture
Ms. Barbara Glenn, on behalf of the Federation of Animal Science Societies
Rebecca Goldburg, Ph.D., on behalf of Environmental Defense
Michael Hansen, Ph.D., on behalf of Consumers Union
Susan Harlander, Ph.D., on behalf of Bionational Consultants
Mr. Arvid Hawk, on behalf of the National Grain and Feed Association
Jason Hlywka, Ph.D., on behalf of Cantox Health Sciences International
Ms. Diana Jackson, on behalf of the Alliance for Bio-Integrity
Ms. Susan Keith, on behalf of the National Corn Growers Association
James Lamb, Ph.D., on behalf of BBL Sciences
Mr. Joseph Mendelson, on behalf of the Center for Food Safety
Ms. Anne Munoz-Furlong, on behalf of the Food Allergy Network
Earl Nestmann, Ph.D., on behalf of the Grocery Manufacturers of America
Ms. Katherine Ozer, on behalf of the National Family Farm Coalition
Barbara Petersen, Ph.D., on behalf of Novigen Sciences and Larry Somerville, Ph.D. on behalf of Aventis CropScience, USA LP
Michael Phillips, Ph.D., on behalf of the Biotechnology Industry Organization
Leah Porter, Ph.D., on behalf of the American Crop Protection Association
Ms. Elizabeth Rice-Arnold, on behalf of the Institute of Science, Technology and Public Policy
Jane Rissler, Ph.D., on behalf of the Union of Concerned Scientists
Lloyd Rooney, Ph.D., on behalf of Texas A&M University
Mr. David Senter, on behalf of the American Corn Growers Association
Ms. Margaret Wittenberg, on behalf of Whole Foods Market
Larry Williams, Ph.D., on behalf of Duke University Medical Center
Jupiter Yeung, Ph.D., on behalf of the National Food Processors Association

Written statements were received from:
Alliance for Bio-Integrity
American Association of Cereal Chemists
American Crop Protection Association
Aventis CorpScience
Mr. Bill Freese
Biorational Consultants, Inc
Blasland, Bouck & Lee, Inc.
BRT, Inc.
Cambridge Environmental, Inc.
Consumer Policy Institute/Consumer Union
Corn Refiners Association
Texas A&M University
Federation of Animal Science Societies
Biotechnology Industry Organization
Environmental Defense
Greenpeace
Harvard Center for Risk Analysis
International Consumers for Civil Society
Medallion Laboratories
Monsanto
National Coalition Against the Misuse of Pesticides
National Corn Growers Association
National Food Processors Association.
National Grain and Feed Association
North American Millers Association
Science for Organizations
United States Department of Agriculture
University of Nebraska
University of Illinois

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 issues pertaining to an assessment of scientific information concerning StarLink™ corn. Advance notice of the meeting was published in the *Federal Register* on November 7, 2000. The review was conducted in an open Panel meeting held in Arlington, Virginia, on November 28, 2000. The meeting was chaired by Stephen Roberts, Ph.D. Mr. Paul Lewis served as the Designated Federal Official. John Kough, Ph.D. (EPA, Office of Pesticide Programs), and Mr. William Jordan (EPA, Office of Pesticide Programs) discussed issues related to allergenicity, sensitization and dietary exposure. Karl C. Klontz, M.D., M.P.H. (Center for Food Safety & Applied Nutrition, Food & Drug Administration) and Carol S. Rubin, D.V.M.,

M.P.H. (National Center for Environmental Health, Centers for Disease Control and Prevention) summarized report of incidents. In this report, Cry9C refers to the protein while cry9c refers to the DNA molecule.

CHARGE

Allergenicity

1. Based on your review of the currently available data, how would you assess the likelihood (high, medium, or low) that the Cry9C protein is a food allergen? Please explain the basis for that conclusion.

Sensitization

2. Assuming the Cry9C protein has the potential to act as a human allergen and taking into account the limited duration (four years) and amount of exposure (no StarLink™ corn produced after 2000) to Cry9C in the food supply, how would you assess the likelihood (high, medium, or low) that the use of StarLink™ corn in making processed food has resulted in the sensitization of some individuals to the Cry9C protein? Please explain the basis for that conclusion. What difference would it make if the amount in the food supply is one or two orders of magnitude lower or higher?

3. The submission from Aventis CropSciences contends that "[i]t is unlikely that a protein, which is present at low levels in the diet, would become an allergen. . . . Allergic responses are not induced by . . . minor components, but are specific for a few usually highly expressed proteins." (Submission, p.21) Aventis contends that Cry9C represents less than 0.0129% of the corn protein, and therefore that it is very unlikely to become a food allergen. Please comment on the scientific basis for this approach to evaluating the potential allergenicity of a protein.

4. Please comment on the relevance of the Bernstein, et al. study on dermal and inhalation sensitization to microbial *Bacillus thuringiensis* pesticide products to sensitization and allergenicity of the Cry9C protein in food.

5. Please comment on the CDC and FDA analysis of reports from individuals who claim to have experienced adverse effects after consuming food that might have been made from StarLink™ corn?

Exposure Estimates

6. Please comment on EPA's methodology for estimating dietary exposure to the Cry9C protein, especially on whether the upper bound estimates are meaningful given corn processing pathways, processed food distribution pathways, and individual consumption patterns.

7. The submission from Aventis CropSciences contends that "[i]n the most conservative approach to safety assessment of the Cry9C protein, the reasonable worst case dietary exposure to the Cry9C protein per day is compared to the amount of peanut allergen required to elicit a clinical response in peanut sensitized individuals." Please comment on whether the comparison of the levels of potential human exposure to Cry9C protein with the levels of peanut allergen causing allergic symptoms in peanut-sensitive individuals is a reasonably conservative approach to assessing the potential risks of Cry9C protein.

Overall

8. Based on your review of the currently available data, how would you assess the overall probability (high, medium, or low) that the likely levels in the US diet of Cry9C protein are sufficient to cause significant allergic reactions in the exposed population? Please explain the basis for that conclusion.

9. Please indicate the priority that should be given to obtaining the following types of additional information for the purpose of improving the scientific basis of assessing the potential allergenic risk of the Cry9C protein:

- Data on the impacts of different processing methods on the level of Cry9C protein in processed food;
- Data on the levels of Cry9C protein found in processed food;
- Data on the extent of mixing of StarLink™ corn grain with StarLink-free corn grain;
- Data on the presence of specific antibodies in individuals either who claim to have experienced adverse effects after consuming food that might have been made from StarLink™ corn or who have significant occupational exposure to StarLink™ corn or corn products; and
- Monitoring of reports from the medical community for individuals who claim to have experienced adverse effects either after consuming food that might have been made from StarLink™ corn or from occupational exposure to StarLink™ corn.

DETAILED RESPONSE TO THE CHARGE

The specific issues to be addressed by the Panel are keyed to the Agency's background document "EPA Preliminary Evaluation of Information Contained in the October 25, 2000 Submission From Aventis CropsScience", dated November 13, 2000, and are presented as follows:

Allergenicity

1. Based on your review of the currently available data, how would you assess the likelihood (high, medium, or low) that the Cry9C protein is a food allergen? Please explain the basis for that conclusion.

The Panel agreed that there is a medium likelihood that the Cry9C protein is a potential allergen based on the biochemical properties of Cry9C protein itself – not its levels in the food supply. Relative to the characteristics of known food allergens, there is no evidence disproving the potential allergenicity of Cry9C. In the Panel's discussion, the following criteria were used to assess Cry9C as a potential allergen. To have a high likelihood of allergenicity, the protein could be expected to have an amino acid sequence similar to known allergens and three or more of the characteristics listed below. The evidence indicates that no amino acid similarity exists between Cry9C and known food allergens; however, not all food allergens or other known allergens have been sequenced. A medium likelihood of allergenicity would have four to five of the listed characteristics, and a protein with a low probability would have one to three of the following characteristics based on data presented to the Panel:

(1) The protein is relatively resistant to acid treatment.
(2) The protein is relatively resistant to protease digestion.
(3) The protein is in the general molecular weight range for an allergen (10 – 70 kD).
(4) The native protein is probably a glycoprotein.
(5) The protein [isolated or in corn] induces an immunologic response in Brown Norway rats.
(6) The protein may be found intact in the bloodstream after oral feeding in the rat model.

Although there is no definitive evidence of the validity of the above criteria, these factors are currently considered to be risk factors for allergenicity (as discussed at the February, 2000 SAP meeting). No single, or two or three criteria, was considered to be adequate to declare the protein a potential allergen, but Cry9C met, to some degree, all of the above. Based on the data submitted since the last SAP meetings, no new data were presented which provided any convincing evidence that Cry9C potential allergenicity was reduced. Taken together, the Panel concluded that Cry9C protein has a medium probability of being a potential allergen.

With respect to criteria #1 and #2 regarding simulated digestibility studies, the following points were made. There was a range of protein resistance based upon pH determinations at 1.2, 1.5 and 2.0 which does not take into consideration the matrix of the Cry9C food source. Nor were the "fasting" values considered to be relevant, as most consumers will not be exposed to Cry9C in a fasted state. The normal population has a relative higher gastric pH value than the pH 1.2 or 1.5 shown to be necessary for Cry9C degradation. In summary, circumstances in which Cry9C digestion was observed do not mimic the physiological state of the host. *In vitro* assessments were not conclusive, but were simply suggestive of allergenicity. It was further noted that the Cry9C transgene protein had been modified to increase protease resistance and therefore would be expected to act differently than the normal wild-type Cry9C protein. This is discussed in more detail in response to criteria #4.

There was discussion relating to degradation versus denaturation and their role in food allergy. Evidence was presented that in extensively hydrolyzed milk formula, residual peptides can induce an allergic response in approximately 10% of cases. In addition, antigen processing by antigen presenting cells (APC) cannot be assessed, as we do not know what APCs or what

proteolytic mechanisms are being performed by localized APCs in the gastrointestinal tract. To what extent this will contribute to a protein being recognized as an allergen is unknown.

With respect to criterion #3, transgenic Cry9C protein, with an apparent molecular weight (MW) of 68 kD and a fragment of approximately 55 kD, is well within the size range of known food allergens.

With respect to criterion #4 regarding the assessment of native Cry9C, the data presented have been developed with the modified transgenic and not the wild-type protein. Data are not currently available to establish with certainty whether or not the wild type protein is glycosylated. Information presented at the February 2000 SAP meeting indicated a discrepancy in molecular weight between wild-type and transgenic products that could be interpreted as evidence for glycosylation.

With respect to #5, the oral or intraperitoneal administration of Cry9C provoked an immunological response in the Brown Norway rat. However, the Panel cautions that the Brown Norway rat is not regarded as a validated animal model for food allergy responses. Non-allergenic food protein sources have not been adequately compared to known allergens in their ability to induce immunologic responses in this (or any) animal model.

With respect to #6, there appeared to be a transport of the intact protein across the mucosal epithelial barrier, as evidence was presented earlier that Cry9C may be found in the blood stream after oral feeding studies in the Brown Norway rat.

Other factors that were considered by the Panel in reaching their decision include the following. Prevalence and frequency data for allergens are generally unknown; however, documented corn allergy is rare, although corn is a major food source. In corn, Cry9C is considered a neoantigen, and should be addressed in clinical situations where it is suspected of inducing a reaction by determining Cry9C-specific IgE and IgG levels and possibly clinical responses to challenges with Cry9C containing food sources.

It was noted that all allergens are proteins/glycoproteins (questions remain on the relevance of carbohydrate IgE-binding epitopes); however, not all proteins are allergens. Nor are all enzyme resistant and/or heat stable proteins allergens. The opposite is also true; not all heat labile or enzyme-degraded proteins can be considered as non-allergenic. Of the multiplicity of proteins in ingested foods, relatively few have been identified as food allergens. Non-allergens have not been characterized to the same extent as known allergens. What must be taken into consideration is that a food allergen is defined as a molecule that will induce an IgE response.

The true prevalence of adverse reactions to foods, including IgE-mediated allergic reactions, is unknown. In the United States, about one-third of families believe some member to be affected by an adverse food reaction (Sloan, 1986). Data from several studies (Burks, 1993; Bock, 1987) estimate that 6-8% of children and 2 – 2.5% of adults have an immunologic

mediated reaction to foods based on clinical history and double blind placebo controlled food challenges. This suggests that children are the population at most risk for the introduction of novel food proteins.

The RAST data, based on proposed corn-specific IgE, was considered to be inconclusive for determining corn related allergy as no clinical history or data reflecting the allergic state of the individual serum used was provided. In the RAST data provided for determining cross-reactivity to other allergens, only a limited number of food allergens were assessed and no inhalant allergens were included. Concern was raised about the different extraction and solubilization methods used to isolate wild-type and transgenic corn protein for the inhibition studies. It was recognized that it would be difficult to determine what serum sources investigators should use to determine Cry9C-specific cross reactivity as no Cry9C specific IgE is yet available. There is a potential for shared epitopes among homologous Cry proteins based on their amino acid sequence homology. Consideration was given to linear versus conformational epitopes. It was noted that airborne allergens may have more conformational epitopes; however, in food allergy studies it has been suggested that conformational epitopes are associated with a transient response while the linear epitopes are associated with long-lasting allergy. It was stressed that IgE binding does not necessarily correlate with a clinical response and that all food allergens have to be adequately characterized to make definitive statements on cross-reactivity.

The Panel members were uncomfortable with the available data; there was an expectation of more antigenicity/allergenicity data based upon prior SAP meeting discussions and recommendations. There was no Cry9C-specific IgE determined in exposed populations. The Garst Seed Company data based on information provided at the February, 2000 SAP meeting and the Bernstein et al study did not measure Cry9C-specific IgE. The IgE-binding proteins were not identified. However, it was pointed out that individual serum from these potentially Cry proteins exposed populations are sources for examination of Cry-specific IgE. These serum sources could be used to assess IgE binding to Cry9C proteins or protein fragments by SDS-PAGE/immunoblot analysis (i.e., Western Blot), a highly sensitive method for detection of native and denatured IgE-binding proteins. These points were made in earlier SAP meetings, as were further immunoreactivity (IgG and IgE antibody responses) results in the animal models, and have not been fully addressed.

Sensitization

2. Assuming the Cry9C protein has the potential to act as a human allergen and taking into account the limited duration (four years) and amount of exposure (no StarLink™ corn produced after 2000) to Cry9C in the food supply, how would you assess the likelihood (high, medium, or low) that the use of StarLink™ corn in making processed food has resulted in the sensitization of some individuals to the Cry9C protein? Please explain the basis for that conclusion. What difference would it make if the amount in the food supply is one or two orders of magnitude lower or higher?

The Panel concluded that the StarLink™ corn Cry9C *Bt*-pesticidal protein should be classified as having low probability to sensitize some individuals to Cry9C protein. Because no single factor is completely predictive of allergenicity and no records of Cry9C human sensitization exist as yet, there can be no final proof that Cry9C is or is not a food allergen. However, the apparent low level of Cry9C protein entering the human diet make it a low likelihood that StarLink™ corn has resulted in sensitization of some individuals to the Cry9C protein. The Panel believes that there would be an enhanced risk if the amount of Cry9C in the food supply would increase by orders of magnitude, whereas lowering the levels makes sensitization less probable.

The Panel has seen no definitive human epidemiologic/survey data suggesting that Cry9C will promote allergic sensitization. The duration of exposure to Cry9C is uncertain, but it may be too short to promote a state of sensitization. The CDC/FDA's case reports of reports on adverse effects after consuming corn products that may or may not have contained StarLink™ corn are inconclusive relative to Cry9C induced sensitization. There are no ELISAs, RAST, skin prick tests or DBPCFC evaluations (Yunginger and Adolphson, 1992; Metcalfe et al., 1996; Bock et al., 1977) on which to base conclusions. The Panel provided a more detailed explanation in its response to question 5.

It is unlikely that the US population has had a long exposure to *cry9c* genes via Bt based microbial products and/or grain dusts. There may be exposure to the cry9 class genes of *B. thuringiensis* in general, but the Bt subspecies that produces Cry9C protein seems unique. Cry9C possesses an amino acid sequence homology of 60-70% relative to Cry9B.

An underlying issue is the need for reproducible, validated methods for analyzing Cry9C levels in processed foods and intermediates, as distinct from PCR methods that identify DNA sequences. Because protein denaturation and/or degradation may produce allergenic epitopes (Hefle, et. al, 1996), these methods need to be more exhaustive than those used for detection of the Cry9C protein in the raw corn. In order to become an allergen, various components of the immune system must encounter significant portions of the protein in antigen presenting cells. The gut wall barrier must be crossed via receptor-mediated and/or endocytosis or other pathways. Based on current knowledge, Cry9C represents a level of approximately 12.9 ppm in StarLink™ corn with yet lower dietary levels after food processing. Therefore, the probability that StarLink™ corn has resulted in the sensitization of individuals to the Cry9C protein is low.

The processes that regulate natural sensitization in humans to any antigen are not well understood. In experimental model systems, several factors are essential including: (1) the critical role of T cell recognition of antigen; (2) the importance of distinct dendritic cells that reside in the gastrointestinal tract and direct T cells to react with the antigen; and (3) the importance of adjuvants or immune boosters that prime the immune system for a protective or hypersensitive response. In humans, the development of sensitization varies widely according to age, family history of atopy, genetic predisposition, disease presentation, and the type of offending food servings (Businco et al. 1999; Taylor et al. 1989). The lower G.I. tract, the

respiratory tract and the tonsils are believed to be the first sites of sensitization. The mechanisms of action in these processes are not known but it is generally believed that antigen sensitization is dependent upon dose and the timing of exposure. In rodent experiments, antigen exposure of younger animals induces greater hypersensitivity than in older animals. Optimal dose-response studies indicate that lower doses of systemic antigens are associated with hypersensitivity rather than tolerances.

While there are some data on the minimum dose required to trigger an allergic reaction in previously sensitized individuals, there is virtually no information on the dosimetry of sensitization in humans. For example, Businco et al., (1999) reported that the oral dose of beta-lactoglobulin causing sensitization in humans is between 1.0 ng and several milligrams. Current animal models address an IgE-mediated reaction relevant for the provocation phase only. Although available, rodent models are not yet regarded as valid models for extrapolation to sensitization in human food allergy. It is also unlikely that any animal model will reliably predict the equivalent of human clinical reactivity. In humans, a critical period is the first two years of life when the gastrointestinal immune system is relatively pre-disposed to allergic responses (van Halteren et al. 1997). Study of infant diets is therefore the highest priority.

It is accepted that during the sensitization process, multiple exposures at sufficiently high amounts are needed in order to induce B lymphocytes to synthesize antigen-specific IgE antibodies, probably with the aid of helper T lymphocytes (Eigenmann and Sampson, 1994; Taylor et al., 1989). The IgE antibodies attach to specialized cells, mast cells, which are present in tissues (e.g., gut wall) and basophils which are present in blood. Upon a subsequent renewed exposure to a much lower dosage level of the allergen, the allergen cross-links IgE molecules on the surface of the sensitized mast cell or the basophil membrane. This cross-linkage triggers release of mediators of allergic reactions. Taken together, it is therefore reasonable to determine the actual level of Cry9C derived antigens in various corn-derived foods and using this information to estimate the risk of sensitization. It is warranted to accurately determine the level of Cry9C in the infant's diet, since children exposed to Cry9C may be more sensitive than adults.

Concerns are raised about the methods used to determine the level of Cry9C in transgenic StarLink™ corn and processed foods. Cry9C DNA has been detected in processed food; however, DNA *per definition* is not toxic and has no history of anticipated allergenicity. It appears that the determination of the protein level is based on immunological detection with poly- and/or monoclonal antiserum against the native full length Cry9C protein (e.g. Western blots and ELISAs). Clearly Cry9C is denatured, at least in part, in processed food. The immunological detection method may in fact be underestimating the concentration of Cry9C due to the failure of the anti-serum to recognize degradation products. This is particularly important since an allergic response may be triggered by degradation products that are not measured with the employed quantification techniques. The difficulty with this area is that there is not a validated analytical methodology that will give reliable results at the levels of Cry9C likely to be detected. On the other hand, the amounts of denatured Cry9C protein, as well as their fate found upon downstream processing should be immunologically further analyzed. In addition,

denaturation by heat or partial proteolysis may uncover new epitopes (Hefle et al., 1996).

3. The submission from Aventis CropSciences contends that "[i]t is unlikely that a protein, which is present at low levels in the diet, would become an allergen. . . . Allergic responses are not induced by . . . minor components, but are specific for a few usually highly expressed proteins." (Submission, p.21) Aventis contends that Cry9C represents less than 0.0129% of the corn protein, and therefore that it is very unlikely to become a food allergen. Please comment on the scientific basis for this approach to evaluating the potential allergenicity of a protein.

The Panel concluded that although most allergens account for >1% of the protein content in an allergenic food, there was a limited predictive value of knowing that Cry9C only accounts for 0.0129% of corn protein. Furthermore, significant issues were raised concerning the validity of the Cry9C protein determination methods when assessing potential allergenicity.

The factors that determine the allergenicity of a protein are not completely understood. Although several properties of proteins have been contended to be important in eliciting allergic responses (e.g., their relative level in a food), it was emphasized that the answer to this question is uncertain. A large amount of experimental work has focused on how an allergen triggers an allergic response and the mechanisms by which allergic antibodies (IgE) are generated. However, the properties that make a protein an allergen remain to be fully examined. Review of the literature concerning the limited number of food allergens that have been identified and characterized to date indicates that food allergens are typically proteins that represent > 1% of the food's total protein. In addition, it was pointed out that although eight foods account for ~ 90% of allergic responses, the number of foods that have been reported to trigger allergic responses is much higher. For example, it is sometimes necessary to test ~50 different foods by skin prick testing, and the number of food allergen extracts that are commercially available exceeds 100. Most food allergens have not yet been characterized. Furthermore, it is possible that allergen expression levels in foods that account for uncommon allergic responses may in fact be lower than 1%. The finding that allergens account for >1% of the protein in common allergic foods does not rule out a role for lower abundance proteins. Only experimental challenge protocols in humans and laboratory animals and a further biochemical and molecular elucidation of other food allergens will address this issue.

The Panel was concerned that the methodology used to determine the concentration of Cry9C protein may be an inadequate approach for estimating biologically active Cry9C protein in exposed humans. The extraction of Cry9C has not been optimized nor standardized, and the solubility of the protein has not been fully assessed. The extraction times reported in studies were of short duration, and may not reflect the total Cry9C protein content. Concerns were also addressed about employment of anti-serum raised against the native full-length protein in ELISA and Western blot assays since these methods may not detect a considerable number of potentially allergenic epitopes. This is compounded by the use of a monoclonal detection antibody in the ELISA; by definition this reagent will only recognize one Cry9C epitope. This is particularly

important since the allergic response may be triggered by Cry9C protein degradation products. Consideration should be given to other techniques used to quantitate proteins. Additionally, it was pointed out that the ELISA may only recognize conformational (non-linear) epitopes which may not be present in processed, denatured, or degraded Cry9C. Given these uncertainties, there was consensus that the determination that Cry9C accounts for <0.0129% of corn protein may possibly be underestimating the concentration of Cry9C that is capable of eliciting an allergic response.

Finally, the Panel evaluated the statement that allergic responses are triggered by abundantly expressed proteins following multiple exposures over an extended period of time. While it appears that this is likely to be true for most food allergens, the Panel indicated that this principle has not been formally proven. For example, although most food allergies develop to abundant proteins (e.g., milk proteins), allergic responses to foods that are not clearly in a child's regular diet (e.g., peanut) are also encountered. This suggests that allergic responses can also be triggered by trace proteins following sporadic exposure, especially in young children. Consistent with this possibility, food allergy can be encountered in exclusively breast fed infants. Presumably, breast fed infants are exposed to trace levels of food antigens derived from the maternal diet or have been exposed *in utero*. Furthermore it is known that infants have developed hypersensitivity reactions [primarily non-IgE-mediated] to residual peptides in extensively hydrolyzed infant formulas. Nevertheless, the levels of antigen that promote sensitization in these settings are not known.

4. Please comment on the relevance of the Bernstein, *et al* study on dermal and inhalation sensitization to microbial *Bacillus thuringiensis* pesticide products to sensitization and allergenicity of the Cry9C protein in food.

The article by Bernstein et al., (Bernstein IL, Bernstein JA, Miller M, Tierzieva S, Bernstein DI, Lummus Z, Selgrade MJK, Doerfler DL, Seligy VL. 1999. Immune responses in farm workers after exposure to *Bacillus thuringiensis* pesticides. Environ Health Persp; 107:575-582) examines immune responses in farm workers with low, medium, and high exposures to foliar application of bacterial pesticides containing *Bacillus thuringiensis* to a variety of Bt spore and vegetative extracts, including an extract enriched for Bt pro-δ-endotoxin. Exposure was assumed to be by either the dermal or respiratory route. Ingestion of small amounts of Bt protein would be assumed. No clinical disease associated with Bt exposure was documented. This paper does not examine Cry9C protein by any means. Thus, this paper has no direct relevance to the issue of sensitivity to Cry9C protein in food nor its allergenicity in foods.

This paper did present evidence of sensitization to Bt foliar applications. There was a significant increase ($P<0.05$) in the number of positive skin tests to spore extracts 1 and 4 months after exposure to Bt spray. The number of antibody positive (IgG and IgE isotypes) subjects was significantly greater ($P<0.05$) in high-exposure workers. Some workers already had IgG antibodies to extracts prior to the study suggesting some prior exposure. IgE titers tended to rise 1 month after exposure, consistent with an anamnestic response, again consistent with a prior

exposure.

While there is no data in this paper that addresses Cry9C or similar proteins directly, Bt pro-δ-endotoxin immunological reactivity was encountered in 2 of 123 workers. The components of the endotoxin were not identified. However, the paper does provide a model of a surveillance program to determine relative sensitization and allergic responses to Cry9C and related proteins.

5. Please comment on the CDC and FDA analysis of reports from individuals who claim to have experienced adverse effects after consuming food that might have been made from StarLink™ corn?

Diagnosing food allergic reactions is a clinical exercise that may be assisted by laboratory data. Food allergic reactions may be due to IgE-mediated reactions, which may produce life-threatening reactions, and non-IgE mediated reactions, which generally induce more chronic, often gastrointestinal reactions. In dealing with self-reported reactions, it is well known that patient history often can not be confirmed. In fact, less than 40% of reported food allergic reactions can be confirmed when patients are subjected to double-blind placebo-controlled food challenges [DBPCFC]. In addition, in many instances, adverse reactions to foods are more frequent following publicity about a specific product. Several public commentors at the meeting noted that reports of possible adverse reactions to StarLink™ did not surface until there was publicity about the potential presence of Cry9C in certain corn products. However, this is not surprising; prior to the first reports, it is very unlikely that anyone would suspect that an allergic reaction is due to corn products since they are eaten all the time with no adverse effect.

As noted above, the majority of reported adverse reactions to foods can not be validated when patients are subjected to DBPCFCs. Perceived food allergy is probably 10 times greater than actual food allergy. Nevertheless, it is possible to follow a systematic approach in order to come to the correct diagnosis of food allergy. The clinical history must address several issues:

(1) Description of the food eliciting the reaction.
(2) Description of symptoms elicited.
(3) Quantity of food ingested.
(4) Timing of reaction.
(5) Whether the individual has eaten the food before.
(6) Is the reaction reproducible.

In dealing with IgE-mediated reactions, the presence of positive skin tests or demonstration of food-specific IgE antibodies in the serum is suggestive of clinical reactivity. About 30% - 40% of patients with IgE antibody to a food will ultimately react in a DBPCFC. In non-IgE-mediated reactions, there are no laboratory studies that will identify the responsible food. Ultimately the use of the DBPCFC can determine clinical reactivity.

The Panel was provided with 34 reports that were received by the FDA from individuals purportedly reacting to Cry9C-contaminated corn products. In addition one public commentor provided information on two other cases, which were not believed to be included with the FDA cases. The Agency requested CDC assistance in the evaluation of reported adverse reactions and the CDC has initiated their review of 26 cases. They have classified reports into those that are possible and those that are probable. Possible cases included those where a suspected anaphylactic reaction occurred [dizziness, weakness, or loss of consciousness within 1 hour of ingesting the suspected food product] or any one of the following skin or oropharyngeal symptoms [hives, rash, itching, tingling, swelling] occurred within 12 hours of ingesting the suspect food product, or any GI symptom [vomiting diarrhea, cramping] occurred within 12 hours of ingesting the suspect food and which involved only one individual. The CDC considered cases "probable" if the patient was treated with corticosteroids or antihistamines and/or was diagnosed by a physician as a probable food allergy. The CDC would consider a case "confirmed" if one of the probable cases had evidence of IgG or IgE antibody to the Cry9C protein. The Panel concluded that this is a very reasonable approach, although it did not believe the presence of IgG or IgE antibodies to Cry9C were sufficient to label a case "confirmed." Instead, the Panel believed that given the histories provided plus evidence of IgE antibody to Cry9C, clinical reactivity could be considered "very likely." Utilizing their classification, the CDC has concluded that 11 of the 26 cases reviewed were "probable" allergic reactions. The Panel would urge the CDC to continue their investigation with diligent speed.

Using an approach similar to that adopted by the CDC, the Panel concluded that at least 7 of 34 reporting individuals were considered to have experienced an allergic reaction to the meal in question: #133, #291, #San2578, #475, #480, #Fla0470, and #Blt5197. However, it is not known whether the corn product they ingested contained the Cry9C protein. Supplemental information should be obtained from these patients as outlined above, and samples of the food ingested obtained to determine if the Cry9C protein was present in the corn product in question. More importantly, serum should be obtained from these patients and evaluated for the presence of IgE antibody to Cry9C. One reportedly milk allergic patient experienced an allergic reaction, but had ingested cheese. An additional 7 subjects experienced reactions that were less likely to be allergic reactions, but data was insufficient to completely rule-out the possibility of an allergic reaction. Twenty of the reports were considered to be very unlikely to represent an allergic reaction. Often the timing was not that of an allergic reaction, the reaction occurred during an apparent viral infection, or multiple individuals became ill at the same feeding. Panel members may vary somewhat in their conclusion as to which cases represent true allergic reactions, but there is agreement that a number of these reports are likely to reflect true allergic reactions.

The Panel believes that this group of 7 – 14 individuals represents a real opportunity to determine whether Cry9C is an allergen. Identification of IgE antibodies to Cry9C in any of these patients would answer the question of whether Cry9C is allergenic; defined by evoking an IgE response and whether it can sensitize a person. The Panel concluded that it would not be possible to answer the question with absolute certainty of whether StarLink™ can induce an allergic reaction without challenging at least some of these patients. The DBPCFC done in a

center skilled in the technique is a safe procedure and is considered standard practice in the diagnosis of food allergy. Follow-up evaluation of these "probable" case reports should be the highest priority in the ongoing evaluation of Cry9C allergenicity.

Exposure Estimates

6. Please comment on EPA's methodology for estimating dietary exposure to the Cry9C protein, especially on whether the upper bound estimates are meaningful given corn processing pathways, processed food distribution pathways, and individual consumption patterns.

There are many factors, as will be described below, that contribute to the complexity in attempting to estimate the exposure to Cry9C protein in the diet. Evaluation of these factors to the extent possible indicates that the methodology used by the Agency to estimate exposure is defendable. The Agency's analysis results in an upper bound estimate that is considerably high and could be justifiably reduced if several of the issues cited were incorporated. However, this conservative approach results in an estimate with a significant safety factor.

As noted in the Agency's background document "Aventis and EPA have taken similar approaches to estimating dietary exposure to Cry9C in processed food. The Agency differs from Aventis with regard to: (1) the level of Cry9C in corn, (2) the assumptions about how to handle "buffer corn," and (3) the assessment of impacts of mixing." Estimating the dietary exposure to the Cry9C protein, at best, is a complex issue with many different considerations involved.

There appears to be no difference between the Agency and Aventis in the amount of Cry9C reported in corn. The Agency uses a value of 12.9 ppm of Cry9C protein expressed in StarLink™ corn grain. Aventis reports a value of 0.0129% for the concentration of Cry9C protein in transgenic, sprayed (glyphosate) whole corn expressed as a percentage of the crude (total) protein. Based on an assumed protein content of 10% for corn, the upper end of protein content normally found in corn, these values are the same.

Although both the Agency and Aventis have agreed upon a value of 12.9 ppm for the presence of Cry9C protein in corn, those familiar with the production of corn know that protein content varies depending on geography, climate, and a number of other factors. In different reports furnished to the SAP, values for Cry9C protein varied from about 10.3 ppm to 13.6 ppm. Cry9C protein content probably varies over a range of 10-20% around the 12.9 ppm value. This should be recognized, or better established through long-term field trials, as encouraged by a previous SAP that dealt with quantification of plant-pesticide proteins.

To give an idea of the amount of Cry9C protein that is present in corn, it is useful to convert it to a bushel basis. A corn kernel weighs about 0.3 gm and corn weighs about 56 lbs per bushel. On this basis, there is 0.312 gm of Cry9C protein per bushel of corn, i.e., about the

weight of one corn kernel per bushel.

Through grower surveys, Aventis estimates that the acreage of buffer zones surrounding StarLink™ corn represents 47% of the total StarLink™ acreage in 2000. Three different scenarios were used by Aventis to estimate the percentage of StarLink™ corn entering food channels. Based on research reports on the movement of pollen from a transgenic plot to surrounding corn plots, the Agency estimates that one-third of the buffer acreage will contain grain having the equivalent of the Cry9C protein content of the StarLink™ corn. Corn planted in the buffer zones must be handled in the same manner as the StarLink™ grain. Both of these values probably overestimate the amount of Cry9C protein from buffer acreage entering the food supply. The upper estimate is probably in the range of the Agency estimate, but it could be zero if the buffer corn is pollinated at a time different than the StarLink™ corn. Relatively little data are cited to support either of these estimates. The Agency noted in its background document that the difference between these two approaches does not appear to be significant. This conclusion appears to be justified.

The approach used by Aventis to estimate the extent and impacts of mixing of StarLink™ and non-transgenic corn appear to be suitable if all corn were first stored in large terminal elevators before movement to milling and food processing facilities. In such facilities, the multiple mixing (blending) described by Aventis and subsequent dilution of the StarLink™ corn contribution will most probably occur. The use of this national approach to mixing will tend to overestimate the extent of mixing.

The Agency's approach using individual state production figures to calculate the distribution of StarLink™ corn production as a percentage of the total U.S. corn crop also has limitations. It is highly likely that the upper bound estimates of the population mean exposure are considerably overestimated as a result of this approach to mixing. The absolute worst case acute exposure scenario without mixing will result in an estimated exposure two orders of magnitude higher than the Agency's current upper bound estimate. However, this would be an extremely unlikely and unrealistic scenario.

The most recent USDA data should be used in estimating the amount of StarLink™ corn potentially in the food supply chain. The percentage of StarLink™ corn, varying from a high of 1.5% in Iowa to a low of 0.02% in Michigan, does not take into account that corn identified by the USDA's Stewardship Program (2000 crop year only). Local variations in production, and limited blending that could affect the amount of Cry9C protein in product also are not considered. Based on the Aventis grower survey for the 1999 crop year, the Agency assumed that between 1% and 40% of StarLink™ corn was not directed to animal feed or industrial uses and, thus, could have been misdirected into the human food supply chain. Appropriately, the Agency elected to construct exposure scenarios that used the extremes of this range. It is reassuring that the amount of 2000 StarLink™ corn that is not "tracked" to date by the USDA inventory system (6%) is within the range of the grower survey.

Neither of these approaches adjust the total corn production for varieties that are handled separately for special uses that could not contain StarLink™ corn, except for the potential for trace contamination. There are at least two situations where little or no mixing will occur. These are with white corn (approximately 50 million bushels, where no transgene hybrids have been produced), and contracted hard-endosperm corns (approximately 100 million bushels). Nearly all of this corn is going into the human food chain affecting estimation of exposure.

There is need for a better evaluation of the amount of StarLink™ corn that could be in the food chain. Several different values, differing by about an order of magnitude, for the amount of corn that left the farm before the end of September 2000 were presented during the SAP meeting. These values represents corn that did not go to feed operations and is presumed to be blended with non-StarLink™ corn in the food chain. Better knowledge of the actual amount of StarLink™ corn would enable a more valid estimate of the contamination to be established. In addition, recent information presented at the SAP meeting suggests that some other hybrids may contain Cry9C protein. This impact is unknown. This is a complex issue to which there does not appear to be a simple answer or approach, short of actual sampling of corn as it comes from bins at elevators, mills, and processing facilities. Such sampling would give a better estimate of the extent of mixing of StarLink™ corn and non-StarLink™ corn.

Since no new StarLink™ corn will be added to the base after 2000, an indication of the mixing anticipated between 2000 and 2004 would be of considerable interest. Will the proportions be different from the current estimate? Mixing should be decreasing, but a scenario of what is expected would be highly useful. At this time, testing kits for the presence of StarLink™ are being used for most corn going to elevators and processors. Thus, it is highly doubtful that much additional StarLink™ corn will be entering the food system.

As is entirely appropriate, both the Agency and Aventis count only those ingredients that contain protein after processing in assessing dietary exposure. Thus foods containing corn bran and corn endosperm are counted, while corn syrup, corn oil, starch and other food forms made from corn grain are not counted since they contain virtually no protein.

A major problem is the lack of a validated method for determining Cry9C protein at low levels to be expected in food products. Analytical methods are available for detecting/measuring cry9c DNA and Cry9C protein in corn and fractions produced by dry milling. However, Cry9C protein has not been detected/measured by a validated method that will give reliable results in taco shells or other food products.

One area of concern is the failure to take into account the effects of processing on the Cry9C protein content of food products. Of the U.S. corn crop, less than 20% is used for food. Three types of milling are involved:

a. Wet milling (1,300 million bushels) – products (starch, oil) contain no protein. The protein goes to feed use. Much of the starch goes to non-food uses.

b. Dry milling (160 million bushels). The Cry9C protein content is reduced by 40% by the milling process. The high-Cry9C stream goes to feed uses.
c. Masa production (60 million bushels). Of this, approximately 48 million bushels is white corn that contains no Cry9C protein.

Process	Total food usage (millions of bushels)	Potential exposure to Cry9C protein (millions of bushels containing Cry9C at 12.9 ppm)
Wet milling	1,300	-0-
Dry milling	160	100
Masa	60	12
Total	1,525	112

As a result of this corn utilization pattern, the amount of protein from yellow dent corn, the source of Cry9C, entering the food chain is significantly reduced. Not taking this into account will greatly overestimate the exposure to Cry9C protein.

Two recent reports from Aventis describe processing studies undertaken at Texas A&M University [**ELISA analysis of Cry9C Protein in CBH351 StarLink™ Corn subject to Pilot Scale Alkaline Process** (completed on November 22, 2000) and **ELISA and Western Blot Analysis of Cry9C Protein Present in CBH351 StarLink™ Corn Samples Obtained via Pilot Scale Alkaline Processing** (completed on November 22, 2000)] concerning detection of Cry9C protein in masa, tortillas, and tortilla chips. The first study used a mild protein extraction and an ELISA method using a monoclonal detection antibody. Very low protein extraction and trace detection of Cry9C protein occurred. The second study used a more vigorous protein extraction procedure and an ELISA method with a polyclonal detection antibody. Cry9C protein was detected and measured in masa, tortillas and tortilla chips. Western blot analysis revealed that additional protein was present in a denatured form in the food matrix.

While this study applies to production of tortillas and tortilla chips, it is likely that denaturation of protein occurs during the processing of other components into cereal products such as breakfast cereals, baked goods containing corn (corn breads and muffin) fried foods containing corn, and extrusion cooked snacks. This indicates that the processing of corn into food products results in a reduction of the amount of Cry9C protein detectable or measurable in the final food product. There also is a question about the allergenic potential of denatured protein. Since there is a scarcity of data bearing on this question, and it is possible that new epitopes could be uncovered during denaturation or that potential allergenicity of the denatured protein would not be significantly reduced, it is justified and prudent to use the value of 12.9 ppm Cry9C as the Agency has done.

Acute exposure should be expressed on a per user basis, not per capita. Dietary exposure assessments should have a mechanism to capture the high end of consumption. This is especially important when user days are low, i.e., for infants. Continuing surveys of food intake by

individuals (CSFII) data are suitable for assessing population daily intakes. However, the data base may not capture the small number of infants having severe allergenicity. For this group, much of the diet prescribed involves corn. Thus, these children could have an unusually high consumption rate. Also, there is a need to estimate Cry9C protein dietary exposure over a longer period of time.

7. The submission from Aventis CropSciences contends that "[i]n the most conservative approach to safety assessment of the Cry9C protein, the reasonable worst case dietary exposure to the Cry9C protein per day is compared to the amount of peanut allergen required to elicit a clinical response in peanut sensitized individuals." Please comment on whether the comparison of the levels of potential human exposure to Cry9C protein with the levels of peanut allergen causing allergic symptoms in peanut-sensitive individuals is a reasonably conservative approach to assessing the potential risks of Cry9C protein.

Peanut allergy represents one of the most severe forms of allergic reactions that can lead to death whereas most accidental allergen challenges result in milder clinical reactions, e.g., the oral allergy syndrome. Individual responses to peanut allergens, as with any documented allergen, vary considerably from sensitized individual to individual. There is no data to establish either a sensitization or allergic response threshold level for any food allergen. We simply do not know what characteristics of a protein make it an allergen; what the protein allergen concentration is that results in sensitization or what route and duration of exposure contributes to a protein becoming a food allergen. It is therefore difficult to gain any inference on the risk of Cry9C allergenicity at predicted doses based on comparisons with peanut allergens or any other allergen. Comparative values with respect to potency, frequency of response and the profile range for all food allergens is unknown. The ranking or potency of food allergens has not been fully established. There is an unknown inherent capacity of some proteins to be allergens, which is also dependent upon the genetics of the host. Therefore, it is difficult to extrapolate from one allergen to another at what level an allergen will induce an allergic response.

In the Hourihane et al., study (1997), 100ug of peanut protein is considered to be a representative value for clinical responses to peanuts. This by no means is predictive of all food allergens. At one extreme, milligram quantities may be necessary to obtain a clinical response in some individuals while in others nanogram quantities may induce a clinical response. In the Hourihane study, depending on the subject, the range for allergen challenge varied from 1-50 mg of peanut protein. There is substantial variability in individuals with respect to the time and dose of challenge. Challenge of food-sensitive individuals with a single concentration of an allergen at one period in time may lead to clinical symptoms; however, upon repeated challenges at a later time, may require more or less of the allergen exposure to induce a clinical response. The mechanisms for this variability are unknown, further establishing that both threshold levels for sensitization and allergic responses are unknown and cannot be predicted for any given allergen.

With the potential exposure levels of Cry9C in food products established in the range of 10 mg, more or less, there is no guarantee that a sensitized individual would not react to that

level of Cry9C protein. The ELISA tests have not been standardized with respect to specificity and sensitivity. It is far too general to speculate, based on unknown sensitization to Cry9C, the potential allergic response level that a re-exposure could initiate.

8. Based on your review of the currently available data, how would you assess the overall probability (high, medium, or low) that the likely levels in the US diet of Cry9C protein are sufficient to cause significant allergic reactions in the exposed population? Please explain the basis for that conclusion.

The Panel assessed the currently submitted data and concurred, while not conclusive, that the likely levels of the Cry9C protein in the US diet of provide sufficient evidence of a low probability of allergenicity in the exposed population.

This conclusion was based on taking consideration several factors:

1. The moderate allergenicity prediction of the protein (as per question 1).
2. The low levels of protein expression in corn products.
3. The low levels of estimated exposure in the diet.
4. The accepted conservative approach used by the Agency to estimate exposure levels.
5. The epidemiological data from workers and consumer surveys are not conclusive for allergenicity of Cry9C.
6. The clinical responses reported to date have been inconclusive in establishing allergic reactions to Cry9C. The members are still concerned about follow up of these cases and expressed strong interest and timely follow-up as noted in their response to question 5.

The factors that came into consideration in making this conclusion include the following points. US citizens may have been exposed to *cry9* genes via Bt foliar applications, grain dusts and/or natural exposure to similar naturally-occurring microorganisms found in the environment. However, the truncated and modified *cry9C* gene is considered unique, with no history of agricultural and food use until early 1998. Question 6 of this report discusses the blending and mixing of the Cry9C that inadvertently entered the food system. The resulting dilution results in Cry9C protein in food products within the ppb range. This is at the lower limits of detection for current analytical methodology.

The Agency's estimates of daily intakes are conservative. These levels of Cry9C in food products are substantially less than the level of 1% of the total protein content cited as one of the characteristics of a food allergen.

With respect to allergenic potential of Cry9C, the Panel noted that known allergens possess a MW from 20 -70 kD and Cry9C lies within this range. Also, Cry9C protein has a high to medium probability of exhibiting stability against gastric proteolytic degradation. Although not conclusively proven, the Cry9C protein may be glycosylated in StarLink™ corn. Glycosylation may be important for allergic potential, although the presence or absence of

glycosylation is not a definite indicator for allergenicity. On the other hand, Cry9C has no homology to any known allergens. Also, no immunogenic and minimal to no toxic effects were observed in a 30-day repeated dose study in mice.

Processing of Cry9C contaminated food products demonstrated a denaturation of Cry9C and fragmentation into smaller MW units. Approximately 0.1-0.2% of the original Cry9C protein remained after alkaline cooking, in a study submitted to the Panel. While this suggests a reduction in allergenicity potential, denatured Cry9C protein should be further analyzed immunologically, because denaturation by heat or partial proteolysis may uncover new epitopes (Hefle et al. 1996). The apparent low bioavailability further reduces the likelihood of an antigen presenting cell recognizing fragments of Cry9C proteins remaining after corn processing.

If corn-derived food products contain a highly potent allergen then allergic reactions should appear within a few years. The epidemiological data from workers and consumer surveys are not conclusive as yet. For example, results of a statistically-designed field study of actual Cry9C levels in foods are not available. The presence of specific IgE antibodies in individuals reporting reactions to Cry9C is of great importance to the issue of sensitization to the Cry9C protein. The clinical follow-up of reported complaints should be completed by specific ELISA, RAST or skin prick testing.

9. Please indicate the priority that should be given to obtaining the following types of additional information for the purpose of improving the scientific basis of assessing the potential allergenic risk of the Cry9C protein:

- **Data on the impacts of different processing methods on the level of Cry9C protein in processed food;**
- **Data on the levels of Cry9C protein found in processed food;**
- **Data on the extent of mixing of StarLink™ corn grain with StarLink-free corn grain;**
- **Data on the presence of specific antibodies in individuals either who claim to have experienced adverse effects after consuming food that might have been made from StarLink™ corn or who have significant occupational exposure to StarLink™ corn or corn products; and**
- **Monitoring of reports from the medical community for individuals who claim to have experienced adverse effects either after consuming food that might have been made from StarLink™ corn or from occupational exposure to StarLink™ corn.**

1. Data on the presence of specific antibodies in individuals either who claim to have experienced adverse effects after consuming food that might have been made from StarLink™ corn or who have significant occupational exposure to StarLink™ corn or corn products.

The Panel unanimously concurred that this is the highest priority and most expeditious approach to answer questions regarding the allergenicity of Cry9C and whether sufficient quantities are in the food chain to sensitize individuals. Given the current state of knowledge regarding allergens and the uncertainties of ascertaining the exact amounts of Cry9C in the food chain, this approach could provide "hard evidence" as opposed to speculation on the question at hand. The Panel would encourage the appropriate regulatory bodies to fully evaluate the reported cases of possible reactivity to Cry9C. Further historical information regarding the subjects allergic reaction, additional attempts to positively identify the presence and level of Cry9C in the meal in question, and laboratory data to identify the presence of IgE [and IgG] antibodies in the subjects experiencing the allergic reaction should be obtained as quickly as possible.

2. Monitoring of reports from the medical community for individuals who claim to have experienced adverse effects either after consuming food that might have been made from StarLink™ corn or from occupational exposure to StarLink™ corn.

The Panel felt that the Agency should place the next highest priority on monitoring of reports from the medical community. The Panel felt that the medical community should be informed of the investigation into the allergenicity of Cry9C in corn products. In addition, monitoring reports from the medical community could supplement the cases currently under investigation and could provide additional support for proving or refuting the allergenicity of Cry9C.

3. Data on the levels of Cry9C protein found in processed food.

The Panel felt that the Agency should place next highest priority on obtaining additional data on levels of Cry9C protein in processed food. The difficulty is that there are no validated analytical methodologies that will give reliable results at the levels of Cry9C likely to be detected. The method used in the study by Aventis appears to be a definite improvement, but it has not been validated. The levels of Cry9C protein in processed food will be extremely difficult to measure with any degree of accuracy unless a reproducible and reliable method is validated. Optimization and standardization of protein extraction and detection methods, and studies of matrix effects are necessary. With agreement on optimal detection systems, data indicating that Cry9C is generally not detectable in processed foods or that Cry9C is present at extremely low levels would support the assessment of a low dietary risk from StarLink™ corn. It should also be noted that discussions by the Panel indicated that obtaining reliable information on the level of Cry9C protein for the wide variety of processed food products will be extremely difficult to obtain.

4. Data on the impact of different processing methods on the level of Cry9C protein in processed food

The Panel felt the Agency should place the next highest priority on obtaining additional data on the impact of processing on the levels of Cry9C foods. Data indicating that Cry9C is reduced or eliminated during processing would obviously support a conclusion of a low dietary risk from StarLink™ corn. However, the Panel recognizes that protein fragments, which are not readily detectable with current methodologies, may remain allergenic. Nevertheless, data on processing effects could determine that there are some categories of food products for which it is unlikely that detectable Cry9C exists.

5. Data on the extent of mixing of StarLink™ corn grain with StarLink-free corn grain

As the question is stated, the Agency should place the lowest priority on determining the mixing of StarLink™ corn with non StarLink™ corn. Given the possible variability of mixing for a particular lot of corn, additional data on the average degree of historical mixing would be inconclusive. The Panel encourages the Agency to review comments on the mixing, blending and concentration estimates outlined in other sections of the report.

The references listed below were either cited by the Panel or are being provided to the Agency for their information.

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