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Office of Pesticide Programs, BPPD – H7504C
U.S. Environmental Protection Agency
Room 266A, Crystal Mall 2
1921 Jefferson Davis Highway
Arlington, VA 22202-4501

Branch Chief: Phil Hutton
Attn: Michael Mendelsohn

November 3, 2000

Subject: **Revised updated safety assessment in support of the pesticide petition for a temporary exemption from the requirement of a tolerance for the plant-pesticide *Bacillus thuringiensis* subsp. *tolworthi* Cry9C and the genetic material necessary for the production of this protein in or on all raw plant agricultural commodities.**
Re: **EPA Registration Number 264-669**

Dear Dr. Hutton,

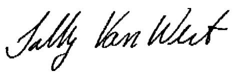
Please find enclosed an additional addendum to the Aventis CropScience USA LP (Aventis) original food tolerance exemption petition for Cry9C corn. This document is a revision of the updated safety assessment that was submitted last week. The revision was made in response to discussions with the Agency on 31 October, 2000.

This revised updated safety assessment was prepared using “worse case” estimates of potential intake of Cry9C protein as a result of corn grown during the 2000 and 1999 growing seasons. Using these “worse case” estimates again there is very strong support for the conclusion that the Cry9C protein meets the Food Quality Protection Act “reasonable certainty of no harm” standard.

On 20 November 1998 Aventis CropScience USA LP (formerly AgrEvo USA Company) submitted a petition proposing to amend 40 CFR 180.1192 to establish exemptions from the requirement of a tolerance for both food and feed uses for the plant-pesticide described above. The EPA has granted to AgrEvo (formerly Plant Genetic Systems (America), Inc.) an exemption from the requirement of a tolerance (22 May 1998) for animal feed uses of this material

Should you have any queries or concerns, Aventis is available at any time to discuss this further and will make industry experts available for such discussions. Please feel free to contact me at (919) 549-2379, or fax (919) 549-3929 or email sally.vanwert@aventis.com.

Sincerely,



Sally Van Wert, Ph.D.
Director, Regulatory Affairs –Biotechnology, North America

Enclosures (EPA Form 8570-1; 3 copies of Volume 1)

Volume #	FIFRA Data Requirement	Title	MRID#
1	Not Applicable	Revised Updated Safety Assessment of StarLink™ Corn Containing Cry9C Protein	

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VOLUME 1 of 1

STUDY TITLE:

Revised Updated Safety Assessment of StarLink™ Corn Containing Cry9C Protein

DATA REQUIREMENT:

Not Applicable

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STUDY COMPLETED ON:

November 3, 2000

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PROJECT IDENTIFICATION:

STARLINK™ 00-02

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this report on the basis of its falling within the scope of FIFRA Section 10 (d)(l)(A),(B), or (C).

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Sally Van Wert, Ph.D.　　Date
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The following information is not subject to the principles of 40 CFR 160, GOOD LABORATORY PRACTICE STANDARDS (FIFRA), as promulgated in *Federal Register*, 54, No. 158, 34067-34704, 17 August 1989. Several studies used as references for this document, however, were conducted in accordance with the appropriate GLP standards as verified by the GLP compliance statements found in these reports.

AUTHOR:

11/3/00

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QUALITY ASSURANCE STATEMENT

REPORT TITLE: Revised Updated Safety Assessment of StarLink™ Corn Containing Cry9C Protein

REPORT IDENTIFICATION: StarLink™ 00-02

This report was audited and reviewed with respect to the study data and the residue files used for the exposure assessment. The data summary tables were derived using an electronic spreadsheet (Excel®). The results of the formulae used in the spreadsheet were independently verified. The information in the text of the report is representative of the data tables; the report contents accurately reflect the data.

11.3.00

Auditor: Beth M. Polakoff, MBA
Director, Regulatory and Business Strategy
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Date

VOLUME 1 of 1

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REVISED UPDATED SAFETY ASSESSMENT OF STARLINK™ CORN CONTAINING CRY9C PROTEIN

I. PURPOSE

The purpose of this document is to determine if, and at what level, Cry9C protein poses a food allergenic potential. The analysis is provided by an updated safety assessment of StarLink™ corn containing Cry9C protein. Specifically, drawing on new data and information, this document provides a comprehensive weight of the evidence analysis of all available information and data.

II. INTRODUCTION

A. Background

StarLink™ corn was registered in 1998 by the US Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) for use as animal feed and for industrial uses (production of ethanol, for example). In granting that registration, EPA concluded that Cry9C protein and related DNA met the safety standard under the FQPA for use in field corn for animal feed use. That is, EPA concluded that “based on the toxicology data cited and the limited exposure expected with animal feed use, there is reasonable certainty that no harm will result from aggregate exposure to the US population, including infants and children” (US EPA Bt Plant-Pesticides Biopesticides Registration Action Document, page IIB18, EPA Scientific Advisory Panel (SAP) website, October 2000 science assessment document). The EPA and the EPA’s SAP were not able to conclude that the Cry9C protein was or was not an allergen (FIFRA SAP Report, Session I – A Set of Scientific Issues being Considered by the Environmental Protection Agency Regarding: Food Allergenicity of cry9C Endotoxin and other Non-digestible Proteins, page 8, June 2000) and, thus, registration for human food use has not yet been granted.

StarLink™ corn is a variety of corn modified through traditional and well-recognized techniques of genetic modification to contain the plant pesticide *Bacillus thuringiensis* (“Bt”) subspecies *toliworthi* Cry9C protein and the genetic material necessary for the production of the protein (DNA). Bt proteins have insecticidal properties and have been used commercially for more than thirty years. Among these products are microbial sprays (Agree, XenTari) with the Cry9B protein, which is highly homologous with the Cry9C protein (Crickmore, et.al., 1998 and Ben-Dov, et.al., 1999). Corn plants with the Bt protein have been widely and safely used for a number of years. These products thus have a long history of safe use.

Pursuant to the registration, StarLink™ corn was planted in 1998, 1999 and 2000. Approximately 10,000 acres were planted in 1998, 250,000 acres were planted in 1999, and 350,000 acres were planted in 2000 out of the approximately 80,000,000 acres of corn planted in the United States in each of those years. Although StarLink™ corn was not registered for use in human food, it now appears that through means not well known, not all of the corn has been kept within the scope of the registered uses (animal feed and non-food industrial uses). The significance to human health of the potential presence of the Cry9C protein and/or the DNA in human food is the subject of this analysis. The analysis relies on the best available data and information and conservative assumptions to assess the potential risks to human health, if any.

B. Approach of the Analysis

Human health assessments typically involve an evaluation of the potential hazard of the material in question and an evaluation of the magnitude of potential exposure to the material. The analysis set forth in this document follows that approach.

First, it identifies the material of potential concern. In the case of StarLink™ corn, the only component of the corn that presents any potential for human health concern is the Cry9C protein and, only then, with regard to the potential for it to cause an allergic reaction in sensitized individuals. The EPA stated that there are no issues relative to the safety of food containing StarLink™ other than the potential allergenicity issue.

Concerning the allergenicity question, this assessment provides a comprehensive review of all available information and data and concludes that Cry9C is not an allergen.

After addressing the data and information pertinent to assessing the question of whether the Cry9C protein is likely to be an allergen, the analysis then turns to an assessment of the potential amount of the protein to which humans might be exposed. This analysis takes into account available information about: (1) the amount of StarLink™ corn planted in 1999 and 2000 and the known or probable disposition of that corn; (2) the quantity of Cry9C protein in corn; (3) the quantity of corn contained in different food products; (4) the fate and disposition of Cry9C protein in food; (5) the quantity of various foodstuffs which contain corn consumed by various population subgroups; and (6) other relevant data.

This assessment considers the risk of adverse allergic responses as a result of a very low level and temporary dietary exposure to Cry9C protein. The strongly supported conclusion is that Cry9C is not an allergen. Furthermore, the assessment strongly concludes that even if Cry9C protein were allergenic, the low level and temporary exposures would neither sensitize individuals nor elicit an allergic response in sensitized individuals. The full basis for these conclusions is set forth below.

C. Context for the Assessment

In order to evaluate properly the potential human health consequences of the presence of Cry9C protein in human food, one must understand how corn is harvested and how it moves through various steps in the distribution chain before it is ultimately used in the production of food for human consumption. With that information, it becomes apparent that there is substantial dilution at each stage of the movement of corn from the farm to the table. To put it differently, the corn from one field or farm is commingled at each stage of the process with corn from other fields and farms.

This section sets forth a brief summary of that information. A full explanation of whole corn handling and grain processing at dry mills is contained in Appendix 1, Corn Handling and Grain Handling Discussion prepared by the North American Millers Association and the National Feed and Grain Association.

Whole Corn Handling Operations from Farm to Elevator

Virtually all farmers harvest corn with a combine equipped with a corn header and transfer the harvested grain from the combine to a truck to deliver either to on-farm storage, a feedlot, or a commercial grain elevator. Farm trucks today typically hold 200 to 800 bushels with the average size about 400 bushels.

When the grain is delivered to a local elevator, it is dumped into a pit. From the pit, the grain is normally conveyed via a bucket elevator to the top of grain storage bins where it is dropped to the bottom of the bin, or onto other grain. Bin sizes at country elevators generally range from 10,000 bushels to 1,000,000 bushels with an average of 70,000 to 80,000 bushels.

Throughout this grain handling process, there is a continuous blending and commingling of the corn from any one farm. The farm truck often carries corn taken from different fields on the farm. When the farm truck arrives at the elevator at harvest, it is frequently one of many trucks in line to dump. In the binning of the grain, the contents of each truck are dumped on top of each other in continuous fashion.

As grain is dropped from the top of storage bins at the elevator, the grain forms an inverted conical shape, as the grain enters at the center and flows out to the sides of the bin. There is a “layering” effect of the grain from each individual truck.

When the grain is drawn from the bottom of the bin, a different flow pattern develops. The grain flowing out will form a "core" in the center. The center portion of the grain bin flows out first, then a cone develops, with the upper portions of the grain flowing out toward the early part of the removal process. As the bin empties, the grain at the sides of the bins starts to flow out of the bottom.

All the truck deliveries used to fill the bin are commingled in the storage/handling process. The degree of mixing of the grain will depend in part on the point at which the truck was dumped. Commingling further occurs as elevators often draw from multiple bins in order to "blend" grain for loading into one transport conveyance to meet quality specifications of different customers.

If an average farm truckload of 400 bushels of pure StarLink™ corn were to be delivered to an elevator and placed into even a small 10,000 bushel bin, a commingling/dilution of that grain on the order of 3 to 5 times is a conservative expectation, with 3 probably a "worst case" situation (Appendix 1, Corn Handling and Grain Handling Discussion prepared by the North American Millers Association and the National Grain and Feed Association).

Grain Processing at Dry Mills

Grain is delivered from elevators to dry corn mills via trucks or rail cars. Trucks typically haul 1,000 bushels with rail cars holding about 3,500 bushels. The initial receiving process is much like that at the elevator, dumping into a pit and elevating grain into storage bins, which hold the grain until it enters the processing stream.

Most dry corn mills are continuous process (rather than batch). Because the grain in a milling operation is being continuously mixed through tempering, milling, and handling, the degree of dilution at any one stage is probably much greater than the factor of three, considered to be the "worst case" at the elevator. Assuming conservatively that there are only seven handling and processing operations, each of which is assumed to dilute the grain by a factor of three, suggests that one truckload of pure StarLink™ corn would be diluted by several orders of magnitude prior to reaching the food processor or consumer.

Wet Milling

Corn is received at wet milling plants via truck, railcar, or barge. Corn is stored at wet mills in a manner similar to dry mills or grain elevators.

The corn wet milling process separates corn into four basic components: starch, germ, fiber and protein. There are five basic steps to accomplish this process. All processes in corn wet milling are continuous (rather than batch).

Incoming corn is inspected and cleaned. It is then steeped in a dilute sulfurous acid solution for 30 to 40 hours. This results in the breaking of the starch and protein bonds. The next step in the process involves coarse grind, which separates the germ from the rest of the kernel. Corn germ is subject to mechanical and solvent extraction to remove oil, which is then refined through degumming, alkali treatment, bleaching, winterization, and vacuum steam stripping deoderization. The remaining slurry consisting of fiber, starch and protein is finely ground and screened to separate the fiber from the starch and protein. Fiber is combined with the water from corn steeping to produce corn gluten feed. The remaining starch and gluten are separated into hydrocyclones. The separated gluten is dried to produce corn gluten meal. The remaining starch is repeatedly washed in fresh water. Water from this washing step flows back through the process countercurrently to the flow of corn. The starch is then converted to sweetners or fermentation products or dried and packaged as starch (Blanchard, 1992). Of the wet milled corn, approximately 60 percent is directed toward sweetner production, 25 percent toward alcohol production, and 15% toward starch production. In the latter case 80 percent is directed toward industrial purposes while the remaining 20 percent is used in food starches (Personal communication, Corn Refiners Association).

As in the case of the dry milling discussion, commingling of corn occurs in the wet milling process. It is estimated that one truckload of pure StarLink™ corn would be diluted by several orders of magnitude, prior to reaching the food processor or consumer. This extensive processing likely leads to, at least, degradation of protein.

D. Safety of *cry9C* DNA and DNA Generally

With respect to the safety of c*ry9C* DNA and DNA in general, EPA has concluded that:

DNA is common to all forms of plant and animal life and the Agency knows of no instance where these nucleic acids have been associated with toxic effects related to their consumption as component of food. These ubiquitous nucleic acids as they appear in the subject plant pesticide have been adequately characterized by the applicant and supports (sic) EPA's conclusion that no mammalian toxicity is anticipated from dietary exposure to the genetic material necessary for the production of the Cry9C protein. (63 Fed. Reg. 28259; 5/22/98).

There is an EPA proposed exemption from the requirement of a tolerance for nucleic acids produced in plants as part of a plant-pesticide (Plant Pesticides; Subject to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA): Proposed Rule, 59 Fed. Reg. 60505; 11/23/94). This proposal states:

> *Residues of nucleic acids produced in living plants as part of a plant-pesticide active or inert ingredient, including both deoxyribonucleic acid and ribonucleic acids, are exempt from the requirement of a tolerance.*

More recently, EPA confirmed its views concerning the safety of nucleic acid in its background materials from the October 18-20, 2000 SAP meeting; Biopesticides Registration Action Document: Bt Plant-Pesticides (http://www.epa.gov/scipoly/sap/).

> *DNA is common to all forms of plant and animal life and the Agency knows of no instance where these nucleic acids have been associated with toxic effects related to their consumption as a component of food.*

In addition, the US Food and Drug Administration (FDA) has also concluded that DNA is generally recognized as safe (1992, FDA Food Policy).

Based on these EPA and FDA statements, the presence of *cry9C* DNA in food is not relevant to the safety assessment of StarLink™ corn because it is recognized as safe.

E. Assessment of Potential Toxicity of Cry9C Protein

Based on the history of the use of Bt microbial pesticides and available toxicity data on Cry9C protein, it is reasonable to conclude that, other than possible allergenicity, there are no toxicity issues related to the food and feed use of Cry9C protein. EPA concurs with that conclusion.

In the final rule establishing the exemption from the requirement of a tolerance for Cry9C protein and genetic material in feed EPA stated:

> *Bt microbial pesticides, containing Cry proteins other than Cry9C, have been applied for more than 30 years in food and feed crops consumed by the US population. There have been no human safety problems attributed to the specific Cry proteins. An oral dose of the tryptic core Cry9C protein of at least 3,760 mg/kg was administered to 10 animals without mortality demonstrating a high degree of safety for the protein. (63 Fed. Reg. 28258; 5/22/98).*

The lack of acute oral toxicity of Cry9C protein is consistent with the lack of toxicity and established safety of other Cry class proteins previously approved for use by the Agency. Furthermore, additional toxicity studies submitted to EPA support this conclusion (*MRID #44734302 and 44734303).* Thus, general toxicity issues are not considered further in this assessment.

F. Assessment of Potential Allergenicity of Cry9C Protein

Given that DNA is recognized as safe, and that there are no general toxicity issues related to Cry9C protein, the only remaining issue relative to the safety of StarLink™ corn is the potential allergenicity of Cry9C protein and the associated level of potential risk.

In regard to the use of StarLink™ corn in animal feed, the EPA concluded that:

> *The Cry9C protein would not likely cause an allergic reaction to man when used in feed corn because; (1) it was not from allergenic sources and (2) the best available information indicates that edible products derived from animals such as meat, milk and eggs intended for human consumption, have not been shown to be altered in their allergenicity due to changes in the feed stock utilized. (US EPA Bt Plant-Pesticides Biopesticides Registration Action Document, page IIB18, EPA Scientific Advisory Panel website, October 2000 science assessment document.)*

This document provides a brief background on food allergy and, drawing on new information and analysis, provides a risk assessment regarding the potential allergenicity for StarLink™ corn expressing Cry9C protein in food. A discussion of the new information relevant to the allergenic potential of the Cry9C protein is also included. Based on a review of all available information and data, this assessment concludes that there is a reasonable certainty that Cry9C protein is not an allergen, and is not likely to become an allergen even if there were long-term consumption.

In an independent review by Dr. S.L. Hefle of the Food Allergy Research and Resource Program, University of Nebraska, Dr. Hefle concluded that "the data shared by Aventis, taken in total, while not conclusive provide evidence that (sic) of low probability of allergenicity of Cry9C" (Appendix 2). A written statement submitted by Dr. S.L. Taylor of the same organization to EPA's SAP (October 20, 2000) supports this conclusion (Appendix 3).

G. Food Allergens and the Use of the Peanut for Comparison Purposes

Food allergy affects 1-2% of adults and 6-8% of children in the United States (Sampson, H.A. *et al.*, 1996; Metcalfe, D.D. *et al.*, 1996). Protecting food allergic patients from unexpected exposure to food allergens is a critical priority. Food allergy assessments ensure that food allergic patients are protected from unexpected exposure to the allergens that might cause them harm. In addition, food allergy assessment evaluates the potential of any new protein to become a new allergen, and to create a newly sensitized population.

In his written submission to the SAP (October 20, 2000), Dr. S.L. Taylor stated that sensitization to foods requires multiple exposures over an extended time period and at a relatively high percentage of total protein content (Appendix 3).

For StarLink™ corn, there is no history of significant consumption, and hence no real potential for allergic sensitization. Furthermore, based on available data and information, the amount of Cry9C protein that could potentially be present in corn products would be present at levels far below those required to cause sensitization. Therefore, it is reasonable to conclude that there are not now and will not be in the future any "at risk" consumers. Furthermore, the EPA has previously concluded that after more than 30 years of commercial use of microbial products containing a variety of Cry proteins, including proteins from the Cry9 class, no allergy has been attributed to Cry proteins (McClintock *et al.*, 1995; EPA, 1999).

Most allergenic proteins are present in levels of 1 to 40% of the total protein of the allergenic food (Metcalfe, D.D., *et al.*, 1996 ; Yunginger, J.W *et al.*, 1997; Li-Chan, E. and Nakai, S., 1989; Murphy, P.A. and Resurrecion, A.P., 1984; Kalinski, A. *et al.*, 1990; Charpentier, B.A. and Lemmel, D.E., 1984; Goldberg, R.B. *et al.*, 1983; Burks, A.W. *et al.*, 1992; Lotan, R. *et al.*, 1975; Crouch and Sussex, 1981). In contrast, there is an extremely low percentage (0.0129%) of the Cry9C protein in StarLink™ corn grain (Table 1) (*MRID #45025701*).

Even lower levels of Cry9C protein might be expected in foods containing corn as an ingredient since, following dry or wet milling, the protein is redistributed into individual commodities. Thereafter food processing exposes the protein to a range of potential degradation procedures which in some instances could completely destroy the protein. In taco shells, for example, no protein was detected (Preliminary Study for Detection of Cry9C Protein in Taco Shells, FIFRA 6(a)(2) report, submitted to EPA on 10/16/00, *MRID #45240203* and Analysis of Taco Shells for Cry9C Protein submitted to EPA on 10/24/00, *MRID #45246402*).

TABLE 1

QUANTITIES OF CRY9C PROTEIN IN PROCESSED COMMODITIES OF STARLINK™ CORN (CBH351) EXPRESSED AS PERCENT OF CRUDE PROTEIN (*MRID #45025701*)

Process	Commodity	Crude Protein (All Types) in Matrix (%)[a]	% Cry9C of the Crude Protein	
			Transgenic Unsprayed[b]	Transgenic Sprayed[c]
	Whole corn	8.9 – 10	0.0116	0.0129
Dry Mill	Composite Grits	7 - 10.3	0.00861	0.0111
	Hull Material	8	0.0130	0.0163
	Meal	7.5 - 9.0	0.00989	0.0118
	Flour	5.2 - 7.8	0.0149	0.0147
	Solvent Extract Germ	12 – 25	0.0345	0.0298
	Crude Oil	0	NA[d]	NA
	Refined Oil	0	NA	NA
Wet Mill	Steepwater Concentrate	41 – 62	0.000034	0.000078
	Hull Material	8	0.00719	0.0146
	Gluten	41 – 60	0.00015	0.00011
	Starch	0.6	NA	NA
	Solvent Extracted Germ	22.6	0.00056	0.00063
	Crude Oil	0	NA	NA
	Refined Oil	0	NA	NA

[a] Range of data from Wolff, I.A. 1982; Ensminger, M.E. *et al.*, 1990; McGregor, C.A. 1994.
[b] Unsprayed = Not treated with Liberty® Herbicide
[c] Sprayed = Post emergent treatment with Liberty® Herbicide
[d] NA - concentration was below limit of quantitation (LOQ) for these samples.

Since allergy to Cry9C protein does not already exist, the extremely low level of Cry9C protein estimated to be consumed using a reasonable, worst case exposure assessment leads to the conclusion that the Cry9C protein present in StarLink™corn is very unlikely to become an allergen.

Peanuts account for the majority of fatal and near-fatal, food-induced, anaphylactic reactions in the United States (Yunginger JW, *et al.*, 1988; Li, X-M, *et al.*, 2000). About 1.5 million Americans (Li, X-M, *et al.*, 2000) are allergic to peanuts. Given the severity, prevalence, and frequently lifelong persistence of peanut allergy, a comparison of the potential allergenicity of a new protein, such as Cry9C protein, with peanuts, one of the most potent known human food allergens, provides an extremely conservative and protective assessment.

III. HAZARD ASSESSMENT

Based on data previously submitted to EPA by Aventis CropSciences USA, LP (Aventis), and based on new information and data described below, it is clear that Cry9C protein has a very low potential of becoming a food allergen. The Cry9C protein does not match the physicochemical characteristics of known food allergens. Results from an oral, 30-day repeated dose study in mice demonstrated no immunological effects at any dose level (*MRID #44734303*). The newly introduced protein in StarLink™ corn has been shown not to alter the endogenous levels of allergens in corn compared to traditional corn varieties (*MRID #44384405*), and the Cry9C protein does not cross-react with sera from patients allergic to other major allergenic foods (Aventis report submitted to EPA on 10/24/00). Finally, the minute levels of Cry9C estimated to be consumed using a reasonable, worst case assessment are orders of magnitude below the levels of allergenic proteins in foods to which people have become sensitized. Based on the following analysis of potential risk, it is clear that the presence of Cry9C protein in food meets the "reasonable certainty of no harm" safety standard under the FQPA; there is a reasonable certainty that Cry9C protein is not and will not become an allergen.

A. Physiochemical Characteristics of Cry9C Protein

To assess the potential allergenicity of a protein, it is useful to compare the physicochemical properties of that protein to known food allergens. Properties such as the structural similarity of a new protein compared to known food and other allergens, stability, and protein levels in food are typically considered (Metcalfe *et al.*, 1996).

Data developed by Aventis support the conclusion that the Cry9C protein has a very low potential of being a food allergen. The data on the parameters to assess the potential for the Cry9C protein to induce sensitization are:

The *Cry9C* gene was obtained from a common soil bacteria, *Bacillus thuringiensis*, which has no known capacity to cause allergies;

1. Cry9C protein lacks structural similarity to any known allergen (food and others). That is, the amino acid sequence of Cry9C protein is not similar to the amino acid sequence in any other known allergen (*MRID #44258109 and 44384404*);
2. Cry9C is not glycosylated in StarLink™ corn in contrast to known allergens (*MRID #44384401*);
3. Cry9C is expressed at extremely low levels (0.0129% of the crude protein in corn grain) relative to known food allergens (*MRID #45025701*); and
4. Based on the results of recent digestibility studies conducted under simulated gastric conditions as defined by the US Pharmacopoeia, Cry9C protein digests within the range of normal human gastric pH and gastric emptying time (*MRID #45114401 and 45114402*).

B. 30-day Repeated Dose Mouse Study

Aventis conducted a 30-day repeated dose study in mice. There were no immunological effects observed at any dose level. Endpoints included an examination of the immune system, blood parameters, reticuloendothelial elements of the bone marrow, and lack of protein binding to villi and crypt cells of the small intestine. Lymphatic tissue of the intestines (i.e., Peyer's patches), the spleen, submandibular glands, mesenteric lymph nodes and thymus were all normal upon microscopic examination (*MRID #44734303*) .

C. Molecular Genetic Effects on Endogenous Corn Allergens

Introduction of the C*ry9C* gene and expression of the Cry9C protein into StarLink™ corn did not alter or enhance the intrinsic allergenic status of corn. Through the technique known as RAST (radioallergosorbent test), it was demonstrated that the serum from corn allergic individuals reacted equivalently to the endogenous allergens in StarLink™ corn and conventional corn. Statistical analysis revealed no significant differences, indicating no differences in the quantity and reactivity of endogenous corn allergens as a result of genetic modification. *(MRID #44384405).*

D. Cross Reactivity

Because it is known that people who are sensitive to known food allergens in food may also react to other food proteins, even without previous exposure, RAST tests were performed to determine if individuals allergic to the well-known human food allergens wheat, rice, buckwheat, soy, peanut, milk, eggs, and shrimp demonstrated cross-reactivity to Cry9C. This study demonstrated a lack of cross-reactivity of the serum from these food-allergic patients to the Cry9C protein, which is consistent with and supports the lack of structural and immunological similarity of Cry9C protein to important food allergens, and provides additional evidence of the low probability that Cry9C is a food allergen. *(*Aventis preliminary report to EPA on 10/24/00, MRID #*45240203*.)

E. Protein Abundance and Potential Allergenicity

It is unlikely that a protein, which is present at low levels in the diet, would become a food allergen (Metcalfe, D.D. *et al.*, 1996; Fuchs, R.L. and Astwood, J.D., 1996; Taylor, S.L., 1992; Hefle, S., 1996; Gendel, S.M., 1998). The induction of all immunological responses is complex, but induction (i.e., becoming sensitized) is clearly dose dependent. A raw food product like soybean flour will contain thousands of different proteins, but most are present at very low concentrations. Allergic responses are not induced by these minor components, but are specific for a few usually highly expressed proteins (Yunginger, 1997; Astwood *et al.*, 1996; Metcalfe *et al.*, 1996).

A rough estimate of consumption of various allergens on an average per serving basis for 2 to 4 year old children is shown in Table 2, and is based on both the total protein and specific major allergen content in foods (Yunginger, 1997; Astwood *et al.*, 1997). This comparison supports the conclusion that important food allergens tend to be relatively abundant in food, and therefore they are consumed at relatively high levels. Conversely, based on this information and the references noted above, it is generally accepted that a protein present at very low levels in food represents a minimal potential for allergic sensitization.

TABLE 2

ESTIMATE OF CONSUMPTION OF VARIOUS ALLERGENS ON AN AVERAGE PER SERVING BASIS FOR 2 TO 4 YEAR OLD CHILDREN

FOOD (SERVING SIZE) [TOTAL PROTEIN]	ALLERGENIC PROTEIN	STRONG/WEAK FOOD ALLERGEN (++, +/-, -)	% OF TOTAL PROTEIN	ALLERGEN /SERVING (MG)
Cow's milk (250 ml)	β-lactoglobulin	++	9	800
[9 g]	α-caseins	++	34	3050
	α-lactalbumin	++	4	350
Soybean (milk 250 ml)	11s glycinin	++	51	2080
[4.2 g]	7s β-conglycinin	++	18.5	770
	Lectin	+/- or -	1	42
	Kunitz trypsin inhb.	+/- or -	2	84
Peanut (butter 32g)	Ara h1	+++	10	800
[8 g]	Ara h2	+++	6	480
Chicken egg (58 g) [white =3.5 g]	Ovomucoid	+	11	385
	Ovalbumin	+	54	1900
Brazil nut (2 g) [1 g]	2s albumin	++	10	100

(Table based on a combination of Astwood J, *et al.*, 1997 and Yunginger, 1997)

In contrast to major food allergens which are typically at high dietary levels (greater than 1% of total protein), the percent of Cry9C protein in StarLink™ corn is approximately 1/80th as abundant (i.e., almost 2 orders of magnitude lower). Therefore, Cry9C protein as produced in StarLink™ corn does not share this important attribute, abundance, with food allergens.

These factors (physiochemical characteristics, immunotoxicity data, molecular genetic effects, cross reactivity and allergen abundance) taken together demonstrate that Cry9C protein and StarLink™ corn share none of the hazard characteristics associated with important food allergens and allergenic foods.

IV. SAFETY ASSESSMENT

A. Potential Dietary Exposure to Cry9C Protein

The potential dietary exposure to Cry9C protein is an important consideration with respect to evaluating the potential for Cry9C to be an allergen. As allergenicity expert Dr. S. L. Taylor, University of Nebraska, noted in a written statement submitted to EPA's FIFRA SAP (October 20, 2000):

> *In order for people to become allergic to a protein they must be exposed to it multiple times over an extended period until they become sensitized. The protein must also be present at a relatively high percentage of total protein content. Most allergenic proteins are present at levels of 1 to 40 percent. Aventis indicates that the Cry9C protein is present in corn grain at 0.013 percent, but any taco shells would contain far less due to the presence of other varieties of corn and the use of other ingredients. (See Appendix 3 for complete statement.)*

Note Dr. Taylor's reference to the Cry9C protein being 0.013 percent of corn grain. As shown in Table 1, Cry9C protein is actually 0.0129 percent of the crude protein in corn grain.

Since multiple exposures over an extended period of time to relatively high levels of protein are known to be required to produce sensitization, the potential for dietary exposure to Cry9C in corn-containing foods is an important consideration. The issue of levels of Cry9C protein potentially present in corn is what we address here.

The analysis has two parts. First, the potential dietary exposures to (or intakes of) Cry9C, per day, from StarLink™ corn were estimated for the US population and selected subgroups, using 1999 and 2000 production data and reasonable worst-case approaches, inputs and assumptions. Second, these estimated potential intakes of Cry9C were compared to levels of peanut allergens in peanut butter. Peanut allergens Ara h1 and Ara h2 were used as a conservative basis for comparison because peanut allergy is prevalent (1.5 million Americans according to Li X.-M. *et al.*, 2000), severe (peanut allergens cause the most fatal and near-fatal anaphylactic reactions, according to Yunginger *et al.*, 1988; Li X.-M. *et al.*, 2000; Bock 1988) and because there is no curative therapy (Li X.-M. *et al.*, 2000).

The approach, including an explanation of the different scenarios, is discussed below. Results of the analyses are presented in Tables 3 through 8.

1. Methodology used to estimate the intake of Cry9C protein

Tables 3 through 8 contain "worst case" estimates of potential intake of Cry9C protein as a result of consumption of corn grown during the 2000 and 1999 growing seasons.

The estimates were derived based on the following information:

- In the year 2000, 0.4% of the U.S. corn supply contains StarLink™ corn.
- In the year 1999, 0.3% of the U.S. corn supply contained StarLink™ corn.
- Corn contains 10% crude protein and the amount of Cry9C protein in the crude protein is 0.0129%.
- The consumption of corn was estimated using Novigen's DEEM™ software. (The DEEM™ software is used by OPP to estimate intake of pesticides.)
- Consumption was estimated on a **per capita basis** (e.g., the entire population whether or not they consumed a corn-containing food) and on a **per user basis** (e.g., estimates for those individuals who consumed a corn-containing food).

Corn is consumed in a variety of different foods. The DEEM™ software breaks that corn into five different components: corn sugar (high-fructose corn syrup, glucose), corn oil, alcohol, corn endosperm and corn bran. Corn sugar, corn oil and alcohol do not contain protein. Therefore, those components were omitted from the analysis. The component of consumption that is called corn endosperm in DEEM™ contains a mixture of different corn food fractions. Corn endosperm contains fractions that do contain protein as well as one fraction, corn starch, that contains little or no protein. The corn protein-containing fractions total 42% based on estimates from the USDA Economic Research Service (National Corn Grower's Association, 2000). Therefore, the estimates of corn endosperm consumption were multiplied by 0.42 to reflect the proportion of corn foods in DEEM™ that contain protein. See Appendix 4 for more detail concerning corn fractions in DEEM™.

2. Dietary intake of those foods potentially containing Cry9C

Consumption data from USDA's 1994-96 Continuing Surveys of Food Intake by Individuals (CFSII) survey were used in the analysis. This is the same database that EPA uses to estimate potential dietary exposures to pesticide residues. All foods with potential to contain protein, and therefore also Cry9C, were included. To determine the daily consumption of foods that potentially contain the Cry9C protein, the CSFII database was queried to calculate the total daily consumption of any foods containing ingredients made from "corn endosperm" (which also includes corn starch) and "corn bran." These are the protein-containing corn fractions.

Daily consumption of foods containing corn protein was determined for the US population and each of the following subpopulations that may be expected to consume high amounts of corn-containing foods: Hispanics, Hispanic children 7 to 12 years of age, Hispanic children 1 to 6 years of age, all US children 7-12 years of age, and all US children 1-6 years of age, on both a *per capita* and *per user* basis. These figures were then multiplied by the exposure factors explained in Section 1, to yield the potential daily dietary intake of Cry9C. This approach is consistent with that used for chemical pesticides. These daily intake estimates are presented in Tables 3 and 4 expressed both as "*per-capita* whole corn consumption" in grams/day *and* "*per-user* whole corn consumption" in grams/day.

Notes accompanying the data tables from the CSFII survey caution that intake estimates based on small cell sizes (i.e., small numbers of observations) tend to be less reliable. According to US government policy (Joint Policy on Variance Estimation and Statistical Reporting Standards for the NHANES III and CSFII Reports, Federation of American Societies for Experimental Biology, Life Science Research Office, 1995), statistically unreliable estimates are to be identified ("flagged") in data tables for the information of users. USDA provides a formula for identifying the minimum number of observations needed for an intake estimate above the 75th percentile to be statistically reliable. Based on application of this formula, we have flagged (footnoted) in Tables 3 and 4 any percentile dietary intake estimates which are statistically unreliable, according to federal government policy for use of the survey data.

Tables 3, 5, and 7 are based on acres planted in crop year 2000 and consumption of corn endosperm and bran. Tables 4, 6, and 8 are based on the acres planted in 1999 and consumption of corn endosperm and bran. As discussed in Section 1, an adjustment of 0.42 was made to remove starch, which contains little, if any, protein.

The amounts of the Cry9C protein consumed per-capita or per-user were calculated by multiplying from left to right across the table. For example, in Table 3, the "amount Cry9C consumed per capita" is obtained by multiplying 0.4% "acres StarLink™ corn in US" times 10% "crude protein in whole corn" times 0.0129% "Cry9C in the crude protein" times 0.42, the "proportion of DEEM™ corn foods that contain protein" times 62.3 "per capita whole corn consumption (per day)."

The amounts of Cry9C protein potentially consumed, in both the 2000 and 1999 "worst case" scenarios are listed below for those subpopulations at the 99.9th percentile with the highest potential consumption "per user":

2000 (See Table 3)		
Hispanic population	99.9th percentile	7.1 μg
All US Children, 7-12 years	99.9th percentile	7.2 μg
1999 (See Table 4)		
Hispanic population	99.9th percentile	5.3 μg
All US Children, 7-12 years	99.9th percentile	5.4 μg

B. Comparison of the Maximum Estimated Intake of Cry9C Protein/Person/Day to Peanut Allergen in Average Serving Size of Peanut Butter

Based upon the average serving size of peanuts, in the form of peanut butter, an individual would consume 32 g of peanuts, of which 8 g/serving is peanut protein (Yunginger, J.W. 1997; Table 1). Of that 8 g of peanut butter protein, the two major peanut allergens (Ara h1 and Ara h2) comprise approximately 1.3 g. Therefore, 1.3 g of these peanut allergens per serving of peanut butter may be considered to represent an established, ongoing level of exposure among the US population of peanut butter consumers. At this established level, the prevalence of peanut allergy in the US is 1.5 million people (Li et al., 2000); this corresponds to about 0.5% of the population.

For purposes of this safety assessment, potential intakes of the Cry9C protein were estimated to evaluate a "Margin of Exposure (MOE)". The MOE was defined by comparing the potential dietary exposure to the Cry9C protein to existing levels of the potent food allergens in the peanut known to be associated with a quantified level of sensitization in the US population. This approach is presented here and in Tables 5-8.

The ratios or MOEs of potential dietary intake of Cry9C per day to the level of peanut allergen in a single serving of peanut butter, are presented in Tables 5 and 6. Table 5 uses the potential Cry9C intakes for the year 2000 as described in Table 3 and Table 6 uses the potential Cry9C intakes for 1999 as described in Table 4.

In contrast to the level of peanut allergen in a single serving of peanut butter, 1.3 g, the maximum level of the Cry9C protein that would potentially be consumed in the year 2000 by the 99.9th percentile corn consumers in the US population is 0.0000072 g/person/day for All US Children, 7-12 years. (See Table 3.) The comparable 1999 number is slightly less (0.0000054 g/person/day. (See Table 4.) The potential dietary exposure to Cry9C protein per day therefore is more than 180,842 times less than the amount of peanut allergens in an average size serving of peanut butter. (See Table 5.) Given that peanut allergens represent arguably the most potent human food allergens, and that the ongoing level of peanut allergens corresponds to a low prevalence of sensitization in the US population (0.5% as discussed previously), it is reasonable to conclude that the likelihood of sensitization associated with the Cry9C protein in StarLink corn is extremely low.

In contrast to the level of peanut allergen in a single serving of peanut butter, 1.3 g, the maximum consumption of peanut butter per day, from the CSFII data, is 186.4 g (US population, 99.9th percentile). Of this, 46.6 g is peanut protein. Of this 46.6 g, the two major peanut allergens, Ara h1 and Ara h2, comprise 7.6 g. This represents the total potential daily exposure to peanut allergens from peanut butter, at the 99.9th percentile. The maximum level of the Cry9C protein that would potentially be consumed in the year 2000 by the 99.9th percentile corn consumers in the US population is 0.0000072 g/person/day for All US Children, 7-12 years. (See Table 3.) The comparable 1999 number is slightly less (0.0000054 g/person/day). (See Table 4.) The potential dietary exposure to Cry9C protein per day therefore is more than 1,057,229 less than the amount of peanut allergens in the maximum consumption of peanut butter per day in 2000 and more than 1,409,639 less than the amount of peanut allergens in the maximum consumption of peanut butter per day in 1999. (See Tables 7 and 8.)

A weight of evidence analysis of available data and information, reinforced by Drs. Taylor and Hefle of the Food Allergy Research and Resource Program at the University of Nebraska, strongly supports the conclusion that Cry9C protein is not likely to be an allergen. However, the analysis discussed above demonstrates that even if the Cry9C protein were an allergen, the potential dietary exposure to Cry9C is so low that sensitization in the population is highly unlikely. Significant MOEs exist.

C. Comparison of Potential Dietary Exposure to Cry9C and Dose of Peanut Allergens Eliciting Responses in Already-Sensitized Individuals

In 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 patients. Recall that consistently higher levels of allergens are required to cause sensitization relative to the amount of allergen required to elicit an allergic reaction in already sensitized individuals.

To assess the amount of protein which is required to elicit a response in already sensitized peanut allergic individuals, a quantitative study using a double blind placebo controlled food challenge approach (DBPCFC) was conducted (Hourihane et al., 1997). In this study, highly peanut allergic patients were challenged with peanut protein at levels ranging from 10 µg to 50 mg to determine the threshold of response. The most highly allergic individuals showed clinical reactions, noted by a physician, at doses of 2 mg of peanut protein/serving (corresponding to 320 µg of Ara h1 and Ara h2 peanut allergens). All subjects with convincing objective reactions had short-lived subjective (reported by the patient) reactions to doses of crude peanut protein as low as 100 µg (corresponding to 16 µg of Ara h1 and Ara h2 peanut allergens), although reactions were mild.

As Tables 3 and 4 illustrate, even using the most conservative scenario and the 99.9th percentile corn consumer (“per-user”), despite their statistical unreliability (as previously discussed), and the highest potential level of exposure to the Cry9C protein, 7.2 µg in 2000 (US All Children, 7-12 years) or 5.4 µg in 1999 (for the same subpopulation), and even if one uses a precautionary principle and assumes that the Cry9C protein was as allergenic as the very potent peanut allergens, these worst case potential consumption levels are less than those which resulted in even mild, subjective symptoms reported by patients already sensitize to peanut allergens.

Perhaps more importantly, the worst case maximum potential human dietary intakes (exposures) to the Cry9C protein, 7.2 µg in 2000 and 5.4 µg in 1999, are also well below the level of peanut allergen exposure that led to mild, objective clinical symptoms of allergy reported by the observing physician in peanut sensitized patients.

Because even the worst case exposure of Cry9C protein is below levels that would result in symptoms for those already sensitized to the most potent food allergen, possible dietary exposure to Cry9C protein is protective for the American population which has not been exposed historically and therefore, is not already sensitized to the Cry9C protein. This reasoning echoes that of both Drs. Steven Taylor and Susan Hefle of the Food Allergy Research and Resource Program.

D. Conclusion of the Safety Assessment

The weight of all the data provides strong evidence of the very low probability of allergenicity of Cry9C protein. (See Appendices 2 and 3.) If however, one assumes that the Cry9C protein has some inherent allergenic potential, based on estimated worst case exposure levels, it is very unlikely that individuals will become sensitized to Cry9C protein. Even if one were to assume that some individuals were, in fact, sensitized, there is little likelihood that there could be a population "at risk" for allergic reactions because estimated exposures are so minimal.

V. CONCLUSIONS

Based on a very conservative assessment of all available information and data, it is clear that Cry9C protein has an extremely low potential of becoming a food allergen. Based on this comprehensive, weight of the evidence analysis of potential risk, it is clear that the presence of Cry9C protein in food meets the "reasonable certainty of no harm" safety standard under the FQPA. This conclusion provides strong support for a time-limited exemption from the requirement for a tolerance for Cry9C protein and the DNA required for its expression in StarLink™ corn.

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TABLE 3

ESTIMATES OF POTENTIAL CRY9C INTAKE FOR CROP YEAR 2000

Population Group	%Acres StarLink ™ Corn in US[1]	% crude protein in whole corn[2]	% Cry9C in crude protein[2]	Proportion of DEEM ™ corn foods that contain protein[3]	Per-Capita whole corn consumption (grams/day) [4]	Per-User whole corn consumption (grams/day)[4]	Amount Cry9C consumed per-capita (g)	Amount Cry9C consumed per-user (g)
US Population								
95th percentile	0.4%	10%	0.0129%	0.42	62.3	68.8	0.0000014	0.0000015
99th percentile	0.4%	10%	0.0129%	0.42	129.1	140.2	0.0000028	0.0000030
99.9th percentile	0.4%	10%	0.0129%	0.42	292.7	312.5	0.0000063	0.0000068
All US Children 1-6								
95th percentile	0.4%	10%	0.0129%	0.42	40.0	41.6	0.0000009	0.0000009
99th percentile	0.4%	10%	0.0129%	0.42	68.0	69.6	0.0000015	0.0000015
99.9th percentile[5]	0.4%	10%	0.0129%	0.42	146.4	146.7	0.0000032	0.0000032
All US Children 7-12								
95th percentile	0.4%	10%	0.0129%	0.42	61.6	64.9	0.0000013	0.0000014
99th percentile	0.4%	10%	0.0129%	0.42	108.9	110.1	0.0000024	0.0000024
99.9th percentile[5]	0.4%	10%	0.0129%	0.42	330.7	331.7	0.0000072	0.0000072

[1] Aventis CropScience data
[2] Aventis CropScience data, MRID # 45025701.
[3] National Corn Grower's Association, 2000.
[4] Based on estimates from USDA CSFII 1994-1996 data
[5] According to Federal Government policy and procedures, this percentile estimate is not statistically reliable. See text.

TABLE 3 (CONT'D)

Population Group	%Acres StarLink™ Corn in US[6]	% crude protein in whole corn[7]	% Cry9C in crude protein[7]	Proportion of DEEM ™ corn foods that contain protein[8]	Per-Capita whole corn consumption (grams/day)[9]	Per-User whole corn consumption (grams/day)[9]	Amount Cry9C consumed per-capita (g)	Amount Cry9C consumed per-user (g)
Hispanic Population								
95th percentile	0.4%	10%	0.0129%	0.42	87.7	98.8	0.0000019	0.0000021
99th percentile	0.4%	10%	0.0129%	0.42	172.3	179.2	0.0000037	0.0000039
99.9th percentile[10]	0.4%	10%	0.0129%	0.42	317.2	328.6	0.0000069	0.0000071
Hispanic Children 1-6								
95th percentile	0.4%	10%	0.0129%	0.42	46.5	48.7	0.0000010	0.0000011
99th percentile[10]	0.4%	10%	0.0129%	0.42	79.1	79.5	0.0000017	0.0000017
99.9th percentile[10]	0.4%	10%	0.0129%	0.42	152.5	315.5	0.0000033	0.0000068
Hispanic Children 7-12								
95th percentile	0.4%	10%	0.0129%	0.42	90.4	91.3	0.0000020	0.0000020
99th percentile[10]	0.4%	10%	0.0129%	0.42	122.4	122.9	0.0000027	0.0000027
99.9th percentile[10]	0.4%	10%	0.0129%	0.42	286.8	286.9	0.0000062	0.0000062

[6] Aventis CropScience data
[7] Aventis CropScience data, MRID # 45025701.
[8] National Corn Grower's Association, 2000.
[9] Based on estimates from USDA CSFII 1994-1996 data
[10] According to Federal Government policy and procedures, this percentile estimate is not statistically reliable. See text.

TABLE 4

ESTIMATES OF POTENTIAL CRY9C INTAKE FOR CROP YEAR 1999

Population Group	% Acres StarLink™ Corn in US[1]	% crude protein in whole corn[2]	% Cry9C in crude protein[2]	Proportion of DEEM ™ corn foods that contain protein[3]	Per-Capita corn consumption (grams/day)[4]	Per-User corn consumption (grams/day)[4]	Worst Case Cry9C consumed Per-capita (g)	Worst Case Cry9C consumed per-user (g)
US Population								
95th percentile	0.3%	10%	0.0129%	0.42	62.3	68.8	0.0000010	0.0000011
99th percentile	0.3%	10%	0.0129%	0.42	129.1	140.2	0.0000021	0.0000023
99.9th percentile	0.3%	10%	0.0129%	0.42	292.7	312.5	0.0000048	0.0000051
All US Children 1 to 6 years								
95th percentile	0.3%	10%	0.0129%	0.42	40	41.6	0.0000007	0.0000007
99th percentile	0.3%	10%	0.0129%	0.42	68	69.6	0.0000011	0.0000011
99.9th percentile[5]	0.3%	10%	0.0129%	0.42	146.4	146.7	0.0000024	0.0000024
All US Children 7-12								
95th percentile	0.3%	10%	0.0129%	0.42	61.6	64.9	0.0000010	0.0000011
99th percentile	0.3%	10%	0.0129%	0.42	108.9	110.1	0.0000018	0.0000018
99.9th percentile[5]	0.3%	10%	0.0129%	0.42	330.7	331.7	0.0000054	0.0000054

[1] Aventis CropScience data
[2] Aventis CropScience data, MRID # 45025701.
[3] National Corn Grower's Association, 2000.
[4] Based on estimates from USDA CSFII 1994-1996 data
[5] According to Federal Government policy and procedures, this percentile estimate is not statistically reliable. See text.

TABLE 4 (CONT'D)

Population Group	% Acres StarLink™ Corn in US[6]	% crude protein in whole corn[7]	% Cry9C in crude protein[7]	Proportion of DEEM ™ corn foods that contain protein[8]	Per-Capita corn consumption (grams/day)[9]	Per-User corn consumption (grams/day)[9]	Worst Case Cry9C consumed Per-capita (g)	Worst Case Cry9C consumed per-user (g)
Hispanic Population								
95th percentile	0.3%	10%	0.0129%	0.42	87.7	98.8	0.0000014	0.0000016
99th percentile	0.3%	10%	0.0129%	0.42	172.3	179.2	0.0000028	0.0000029
99.9th percentile[10]	0.3%	10%	0.0129%	0.42	317.2	328.6	0.0000052	0.0000053
Hispanic Children 1-6								
95th percentile	0.3%	10%	0.0129%	0.42	46.5	48.7	0.0000008	0.0000008
99th percentile[10]	0.3%	10%	0.0129%	0.42	79.1	79.5	0.0000013	0.0000013
99.9th percentile[10]	0.3%	10%	0.0129%	0.42	152.5	315.5	0.0000025	0.0000051
Hispanic Children 7 to 12 years								
95th percentile	0.3%	10%	0.0129%	0.42	90.4	91.3	0.0000015	0.0000015
99th percentile[10]	0.3%	10%	0.0129%	0.42	122.4	122.9	0.0000020	0.0000020
99.9th percentile[10]	0.3%	10%	0.0129%	0.42	286.8	286.9	0.0000047	0.0000047

[6] Aventis CropScience data
[7] Aventis CropScience data, MRID # 45025701.
[8] National Corn Grower's Association, 2000.
[9] Based on estimates from USDA CSFII 1994-1996 data
[10] According to Federal Government policy and procedures, this percentile estimate is not statistically reliable. See text.

TABLE 5

COMPARISON OF DAILY CRY9C INTAKE IN YEAR 2000 AND PEANUT ALLERGENS IN ONE SERVING PEANUT BUTTER

Population Group	Peanut allergen in average 32g serving	Maximum Daily Cry9C consumed per-capita (grams)[1]	Maximum Daily Cry9C consumed per-user (grams)[1]	"Margin of Exposure"[2] per-capita	"Margin of Exposure" per-user
US Population					
95th percentile	1.3 grams	0.0000014	0.0000015	962,845	871,878
99th percentile	1.3 grams	0.0000028	0.0000030	464,642	427,855
99.9th percentile	1.3 grams	0.0000063	0.0000068	204,938	191,953
All US Children 1 to 6 years					
95th percentile	1.3 grams	0.0000009	0.0000009	1,499,631	1,441,953
99th percentile	1.3 grams	0.0000015	0.0000015	882,136	861,857
99.9th percentile	1.3 grams	0.0000032	0.0000032	409,735	408,897
All US Children 7 to 12 years					
95th percentile	1.3 grams	0.0000013	0.0000014	973,786	924,272
99th percentile	1.3 grams	0.0000024	0.0000024	550,829	544,825
99.9th percentile	1.3 grams	0.0000072	0.0000072	181,389	180,842

[1] Consumption Estimates, based on calculations presented in Table 3, are rounded to seven decimals. Therefore, hand-calculated "Margin of Exposures" may be different from those appearing in this table.

[2] "Margin of Exposure" for purposes of this assessment is defined as the amount of peanut allergen/amount of Cry9C. The comparison is relating potential Cry9C exposure to level of allergen in a food known to be associated with quantified level of sensitization in the US population.

TABLE 5 (CONT'D)

Population Group	Peanut allergen in average 32g serving	Maximum Daily Cry9C consumed per-capita (grams) [3]	Maximum Daily Cry9C consumed per-user (grams) [3]	"Margin of Exposure"[4] per-capita	"Margin of Exposure" per-user
Hispanics					
95th percentile	1.3 grams	0.0000019	0.0000021	683,982	607,138
99th percentile	1.3 grams	0.0000037	0.0000039	348,144	334,739
99.9th percentile	1.3 grams	0.0000069	0.0000071	189,109	182,548
Hispanic Children 1 to 6 years					
95th percentile	1.3 grams	0.0000010	0.0000011	1,290,005	1,231,730
99th percentile	1.3 grams	0.0000017	0.0000017	758,347	754,531
99.9th percentile	1.3 grams	0.0000033	0.0000068	393,346	190,128
Hispanic Children 7 to 12 years					
95th percentile	1.3 grams	0.0000020	0.0000020	663,553	657,012
99th percentile	1.3 grams	0.0000027	0.0000027	490,075	488,082
99.9th percentile	1.3 grams	0.0000062	0.0000062	209,154	209,081

3 Consumption Estimates, based on calculations presented in Table 3, are rounded to seven decimals. Therefore, hand-calculated "Margin of Exposures" may be different from those appearing in this table.

4 "Margin of Exposure" for purposes of this assessment is defined as the amount of peanut allergen/amount of Cry9C. The comparison is relating potential Cry9C exposure to level of allergen in a food known to be associated with quantified level of sensitization in the US population.

TABLE 6

COMPARISON OF DAILY CRY9C INTAKE IN YEAR 1999 AND PEANUT ALLERGENS IN ONE SERVING PEANUT BUTTER

Population Group	Peanut allergen in average 32g serving	Maximum Daily Cry9C consumed per-capita (grams) [1]	Maximum Daily Cry9C consumed per-user (grams) [1]	"Margin of Exposure"[2] per-capita	"Margin of Exposure" per-user
US Population					
95th percentile	1.3 grams	0.0000010	0.0000011	1,283,793	1,162,505
99th percentile	1.3 grams	0.0000021	0.0000023	619,522	570,473
99.9th percentile	1.3 grams	0.0000048	0.0000051	273,250	255,937
All US Children 1 to 6 years					
95th percentile	1.3 grams	0.0000007	0.0000007	1,999,508	1,922,604
99th percentile	1.3 grams	0.0000011	0.0000011	1,176,181	1,149,142
99.9th percentile	1.3 grams	0.0000024	0.0000024	546,314	545,196
All US Children 7 to 12 years					
95th percentile	1.3 grams	0.0000010	0.0000011	1,298,382	1,232,362
99th percentile	1.3 grams	0.0000018	0.0000018	734,438	726,433
99.9th percentile	1.3 grams	0.0000054	0.0000054	241,852	241,122

[1] Consumption Estimates, based on calculations presented in Table 4, are rounded to seven decimals. Therefore, hand-calculated "Margin of Exposures" may be different from those appearing in this table.

[2] "Margin of Exposure" is defined as the amount of peanut allergen/amount of Cry9C. The comparison is relating potential Cry9C exposure to level of allergen in a food known to be associated with quantified level of sensitization in the US population.

TABLE 6 (CONT'D)

Population Group	Peanut allergen in average 32g serving	Maximum Daily Cry9C consumed per-capita (grams) [3]	Maximum Daily Cry9C consumed per-user (grams) [3]	"Margin of Exposure"[4] per-capita	"Margin of Exposure" per-user
Hispanics					
95th percentile	1.3 grams	0.0000014	0.0000016	911,976	809,517
99th percentile	1.3 grams	0.0000028	0.0000029	464,192	446,319
99.9th percentile	1.3 grams	0.0000052	0.0000053	252,145	243,397
Hispanic Children 1 to 6 years					
95th percentile	1.3 grams	0.0000008	0.0000008	1,720,007	1,642,306
99th percentile	1.3 grams	0.0000013	0.0000013	1,011,129	1,006,042
99.9th percentile	1.3 grams	0.0000025	0.0000051	524,461	253,503
Hispanic Children 7 to 12 years					
95th percentile	1.3 grams	0.0000015	0.0000015	884,738	876,017
99th percentile	1.3 grams	0.0000020	0.0000020	653,434	650,776
99.9th percentile	1.3 grams	0.0000047	0.0000047	278,871	278,774

[3] Consumption Estimates, based on calculations presented in Table 4, are rounded to seven decimals. Therefore, hand-calculated "Margin of Exposures" may be different from those appearing in this table.

[4] "Margin of Exposure" is defined as the amount of peanut allergen/amount of Cry9C. The comparison is relating potential Cry9C exposure to level of allergen in a food known to be associated with quantified level of sensitization in the US population.

TABLE 7

COMPARISON OF DAILY CRY9C IN YEAR 2000 AND PEANUT ALLERGENS IN DAILY PEANUT BUTTER CONSUMPTION

Population Group	Maximum Peanut allergen in Peanut Butter consumed/day	Maximum Daily Cry9C consumed per-capita (grams) [1]	Maximum Daily Cry9C consumed per-user (grams) [1]	"Margin of Exposure"[2] per-capita	"Margin of Exposure" per-user
US Population					
95th percentile	7.6 grams	0.0000014	0.0000015	5,628,939	5,097,135
99th percentile	7.6 grams	0.0000028	0.0000030	2,716,366	2,501,305
99.9th percentile	7.6 grams	0.0000063	0.0000068	1,198,097	1,122,185
All US Children 1 to 6 years					
95th percentile	7.6 grams	0.0000009	0.0000009	8,767,073	8,429,878
99th percentile	7.6 grams	0.0000015	0.0000015	5,157,102	5,038,548
99.9th percentile	7.6 grams	0.0000032	0.0000032	2,395,375	2,390,477
All US Children 7 to 12 years					
95th percentile	7.6 grams	0.0000013	0.0000014	5,692,904	5,403,435
99th percentile	7.6 grams	0.0000024	0.0000024	3,220,229	3,185,131
99.9th percentile	7.6 grams	0.0000072	0.0000072	1,060,426	1,057,229

[1] Consumption Estimates, based on calculations presented in Table 3, are rounded to seven decimals. Therefore, hand-calculated "Margin of Exposures" may be different from those appearing in this table.

[2] "Margin of Exposure" is defined as the amount of peanut allergen/amount of Cry9C. The comparison is relating potential Cry9C exposure to level of allergen in a food known to be associated with quantified level of sensitization in the US population.

TABLE 7 (CONT'D)

Population Group	Maximum Peanut allergen in Peanut Butter consumed/day	Maximum Daily Cry9C consumed per-capita (grams) [3]	Maximum Daily Cry9C consumed per-user (grams) [3]	"Margin of Exposure"[4] per-capita	"Margin of Exposure" per-user
Hispanics					
95th percentile	7.6 grams	0.0000019	0.0000021	3,998,665	3,549,422
99th percentile	7.6 grams	0.0000037	0.0000039	2,035,304	1,956,936
99.9th percentile	7.6 grams	0.0000069	0.0000071	1,105,558	1,067,203
Hispanic Children 1 to 6 years					
95th percentile	7.6 grams	0.0000010	0.0000011	7,541,568	7,200,881
99th percentile	7.6 grams	0.0000017	0.0000017	4,433,412	4,411,106
99.9th percentile	7.6 grams	0.0000033	0.0000068	2,299,560	1,111,515
Hispanic Children 7 to 12 years					
95th percentile	7.6 grams	0.0000020	0.0000020	3,879,236	3,840,996
99th percentile	7.6 grams	0.0000027	0.0000027	2,865,056	2,853,400
99.9th percentile	7.6 grams	0.0000062	0.0000062	1,222,744	1,222,318

[3] Consumption Estimates, based on calculations presented in Table 3, are rounded to seven decimals. Therefore, hand-calculated "Margin of Exposures" may be different from those appearing in this table.

[4] "Margin of Exposure" is defined as the amount of peanut allergen/amount of Cry9C. The comparison is relating potential Cry9C exposure to level of allergen in a food known to be associated with quantified level of sensitization in the US population.

TABLE 8

COMPARISON OF DAILY CRY9C INTAKE IN YEAR 1999 AND PEANUT ALLERGENS IN DAILY PEANUT BUTTER CONSUMPTION

Population Group	Maximum Peanut allergen from Peanut Butter consumed per day	Maximum Daily Cry9C consumed per-capita (grams) [1]	Maximum Daily Cry9C consumed per-user (grams)[1]	"Margin of Exposure"[2] per-capita	"Margin of Exposure" per-user
US Population					
95th percentile	7.6 grams	0.0000010	0.0000011	7,505,252	6,796,180
99th percentile	7.6 grams	0.0000021	0.0000023	3,621,822	3,335,073
99.9th percentile	7.6 grams	0.0000048	0.0000051	1,597,462	1,496,247
All US Children 1 to 6 years					
95th percentile	7.6 grams	0.0000007	0.0000007	11,689,430	11,239,837
99th percentile	7.6 grams	0.0000011	0.0000011	6,876,135	6,718,063
99.9th percentile	7.6 grams	0.0000024	0.0000024	3,193,833	3,187,302
All US Children 7 to 12 years					
95th percentile	7.6 grams	0.0000010	0.0000011	7,590,539	7,204,580
99th percentile	7.6 grams	0.0000018	0.0000018	4,293,638	4,246,841
99.9th percentile	7.6 grams	0.0000054	0.0000054	1,413,901	1,409,639

[1] Consumption Estimates, based on calculations presented in Table 4, are rounded to seven decimals. Therefore, hand-calculated "Margin of Exposures" may be different from those appearing in this table.

[2] "Margin of Exposure" is defined as the amount of peanut allergen/amount of Cry9C. The comparison is relating potential Cry9C exposure to level of allergen in a food known to be associated with quantified level of sensitization in the US population.

TABLE 8 (CONT'D)

Population Group	Maximum Peanut allergen from Peanut Butter consumed per day	Maximum Daily Cry9C consumed per-capita (grams) [3]	Maximum Daily Cry9C consumed per-user (grams)[3]	"Margin of Exposure"[4] per-capita	"Margin of Exposure" per-user
Hispanics					
95th percentile	7.6 grams	0.0000014	0.0000016	5,331,553	4,732,563
99th percentile	7.6 grams	0.0000028	0.0000029	2,713,739	2,609,248
99.9th percentile	7.6 grams	0.0000052	0.0000053	1,474,077	1,422,937
Hispanic Children 1 to 6 years					
95th percentile	7.6 grams	0.0000008	0.0000008	10,055,424	9,601,175
99th percentile	7.6 grams	0.0000013	0.0000013	5,911,216	5,881,474
99.9th percentile	7.6 grams	0.0000025	0.0000051	3,066,080	1,482,020
Hispanic Children 7 to 12 years					
95th percentile	7.6 grams	0.0000015	0.0000015	5,172,314	5,121,328
99th percentile	7.6 grams	0.0000020	0.0000020	3,820,075	3,804,534
99.9th percentile	7.6 grams	0.0000047	0.0000047	1,630,325	1,629,757

3 Consumption Estimates, based on calculations presented in Table 4, are rounded to seven decimals. Therefore, hand-calculated "Margin of Exposures" may be different from those appearing in this table.

4 "Margin of Exposure" is defined as the amount of peanut allergen/amount of Cry9C. The comparison is relating potential Cry9C exposure to level of allergen in a food known to be associated with quantified level of sensitization in the US population.

APPENDIX 1

CORN HANDLING AND GRAIN HANDLING DISCUSSION

National Grain and Feed Association

APPENDIX 1

CORN HANDLING AND GRAIN PROCESSING DISCUSSION*

Whole Corn Handling Operations from Farm to Elevator

Virtually all farmers harvest corn with a combine with an attached corn header, and transfer the harvested grain from the combine to a truck to deliver either to on-farm storage or a commercial grain elevator. Farm trucks typically hold 200 to 800 bushels with the average size about 400 bushels.

When the grain is delivered to a local elevator, the grain is dumped into a pit covered by an iron grate (which removes large foreign objects). The pit may be able to hold one or more truck loads of grain at a given time. From the pit, the grain is normally conveyed (via belt or drag conveyor) to a bucket elevator which elevates the grain to the top of grain storage bins where it is dropped to the bottom of the bin, or onto other grain. Bin sizes at elevators generally range from 10,000 bushels to 1,000,000 bushels, with an average of 70,000 to 80,000 bushels

When the grain is loaded out of the elevator, it is drawn from the bottom of the bin. The grain flows out of the bin onto a belt or a drag conveyer, and then elevated to again be dumped into a truck, barge or railcar for transshipment to a feeding operation, to a terminal elevator (for additional storage), to a grain processor or to an export location.

From the time of receipt through load-out, there is a continuous blending and commingling of the corn received from individual farmers. The farm truck often carries corn taken from different fields on the farm. Truckloads are dumped successively on top of each other, but the necessary handling, conditioning and management of elevator storage space ensures that individual truckloads lose their identity. Corn that is dried is handled in a different stream through the dryer prior to going to a bin, adding to the commingling process. At some elevators, multiple truck dump pits are combined into one grain stream entering storage. At all facilities, the need to move grain from bin to bin for conditioning of the grain and to open up additional empty bins forces the contents of multiple bins to be commingled into one during handling. Further commingling occurs during load-out as the elevator manager often draws grain from multiple bins to intentionally blend the grain to meet quality specifications for different customers.

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National Grain and Feed Association

As grain is dropped from the top of storage bins at the elevator, the grain forms an inverted conical shape, as the grain enters at the center and flows out to the sides of the bin. There is a "layering" effect of the grain entering the bin. When the grain is drawn from the bottom of the bin, a different flow pattern develops. The grain flowing out will form a "core" in the center. The center portion of the grain bin flows out first, then a cone develops, with the upper portions of the grain flowing out toward the early part of the removal process. As the bin empties, the grain at the sides of the bins starts to flow inward toward the center "core." All the grain deliveries used to fill the bin are commingled in the storage/handling process. The degree of mixing of the grain will depend in part on the point at which the grain entered the bin---near the beginning of the bin-filling process or near the end. The last lot of grain dumped into the bin is likely to have the least amount of commingling in the stream of grain exiting the bin, because the top portion of the grain tends to flow out earlier. Those trucks dumped near the middle of the bin-filling process are commingled most extensively.

If an average farm truck load of 400 bushels of pure StarLink™ corn was delivered to an elevator and placed into a small 10,000 bushel bin, a commingling/dilution of that grain on the order of 3 to 5 times is a conservative expectation, with 3 probably a "worst case" situation. This worst case situation would assume the very minimum number of handlings for drying, conditioning and blending (to meet quality specifications) in the elevator prior to load-out.

Grain Processing at Dry Mills

Grain is delivered from elevators to dry corn mills via trucks or rail cars. Trucks typically haul 1,000 bushels with rail cars holding about 3,500 bushels. The initial receiving process is much like that at the elevator, dumping into a pit and elevating grain into storage bins, which hold the grain until it enters the processing stream.

Most dry corn mills are continuous process (rather than batch). The corn is transferred from the storage bins to a "surge" bin that holds the grain prior to going into a tempering process (where water is added to condition the grain for efficient processing). After tempering, the corn enters the milling process where a series of grinding and sifting operations take place. The germ and the bran are removed from the kernel, and the remaining endosperm portion is reduced to the appropriate size for the product being manufactured. The wide variety of products manufactured includes flaking grits, cereal grits, brewers' grits, corn meal, corn flour, etc.

The various products from milling are transferred into different mill product storage bins depending on intended shipment method. No single bushel goes into any one product bin. The milling of each bushel of corn will create many different particle sizes, each of which goes into a different product bin. From these bins, product may be loaded out in bulk truck or rail or into bags for delivery to a packaging operation or company which may further process or mix the product with other ingredients to produce retail products.

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Each handling process into and out of storage, and each processing operation causes the corn and its products to be diluted further. Through storage, tempering, multiple grinding/sifting operations, transfer into product bins, further processing into retail products, there are at least 7-8 distinct points of dilution during the entire voyage from field to end-user.

Because the grain in a milling operation is being continuously mixed through tempering, milling, and handling, the degree of dilution at any one stage is probably much greater than the factor of three, considered to be the "worst case" at the elevator. However, assuming conservatively that there are only seven handling and processing operations, each of which is assumed to dilute the grain by a factor of three, suggests that one truckload of pure StarLink™ corn would be diluted by several orders of magnitude, prior to reaching the consumer.

* Discussion prepared by Betsy Faga, President of the North American Millers Association and Kendall Keith, President of the National Grain and Feed Association

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APPENDIX 2

LETTER FROM SUSAN L. HEFLE, PH.D., FOOD ALLERGY RESEARCH AND RESOURCES PROGRAM, UNIVERSITY OF NEBRASKA

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IANR

October 11, 2000

Dr. Barbara J. Henry, Ph.D.
Aventis CropScience
Toxicology Department
2 T.W. Alexander Drive
Research Triangle Park, NC 27709

Dear Dr. Henry,

I have reviewed the data summaries that Aventis CropScience has provided to me for assessment of the allergenic potential of Cry9C endotoxin. The paragraphs below summarize my opinion about the data presented to date and what the implications of this data might be.

I was a member of the panel that produced the ILSI-IFBC Decision Tree for the Assessment of Allergenicity of Foods Produced by Genetic Modification (1996), and I feel that Aventis has provided the regulatory agency with a considerable amount of data for use in addressing the question of potential allergenicity of Cry9C. In addition, these data are of the correct type as outlined in the ILSI-IFBC Decision Tree in addressing the issue of potential allergenicity of a novel protein with no allergenic history.

A few notes on biochemical properties before I review the specific allergenicity data:

Cry9C is reported to have a molecular weight of 68.7 kD and a 55 kD band after digestion or degradation. While some in the allergy field have stated that most allergenic proteins are from 10-40 kD in molecular weight, this is no scientific basis for assumption that those lying outside this range would tend less to be allergens. As an example, the major allergen of peanut, Ara h 1, has a molecular weight of approximately 66 kD. Also, ranges of reported molecular weights are highly influenced by the fact that the primary method used to detect allergenicity is by using SDS-PAGE followed by IgE immunoblotting, a denaturing method that is not that precise for measuring molecular weight and reduces proteins to their subunits or smaller peptides rather than revealing their native molecular weights.

In addition, in my opinion the presence or absence of glycosylation is not a definite indicator of allergenic potential. While most food allergens isolated to date have been glycosylated, and in some studies the carbohydrate moieties have been found to be important in IgE binding, there are other food allergens where the carbohydrate portion is not necessary for IgE binding. A basic property of antigens is glycosylation, but the vast majority of antigens are not allergens.

More specific allergenicity testing:

Corn Serum Study - This study as designed and executed does not address the allergenic potential of Cry9C as a novel dietary protein. However, studies of this sort have been suggested by certain scientists in the allergy field as a screen for immunogenic potential. Due to the high prevalence of perceived corn allergy vs. the true prevalence of corn allergy in the U.S. population, the presence of corn allergy should have been documented using double-blind challenges to corn. For food allergy there is a 50-70% false positive rate for in vitro methods of detecting IgE. Also, for this same reason, there is no value in performing general screens of serum for reactivity to Cry9C as has been suggested by certain investigators - in vitro reactivity does not correlate to clinical reactivity in the general population or even in certain atopic populations.

Sequence homology - The finding that there are no matches with 8 contiguous amino acids in Cry9C and gluten or any known allergens is expected. This lack of homology would suggest that Cry9C has no known similarity to any known allergen. I would caution, however, that there are more food allergens identified, isolated, and characterized every day, and that relying on SWISS Prot is not the best way to handle this. Aventis should consider creating its own database and peruse the allergy literature often to add information about epitopes and sequences; a database such as this would be much more complete than SWISS Prot is at any particular time, in my experience. However, I will say that none of the Bt toxins approved to date have any structural similarities with any known allergens and I do not recommend that any further homology testing with Cry9C be done at this time.

Stability to heat and processing - The Cry9C protein appears to be stable to heat to a temperature of 90C for 10 minutes. This would indicate that the protein is stable to heat at fairly high temperatures, but a range of temperature treatments would be more informative. Inclusion of treatments at typical processing temperatures would be useful, although it is unknown if the Cry9C protein would be affected at temperatures other than 90C. There is an ongoing argument about whether or not heat stability can be related to potential allergenicity. In food allergens described to date, most are heat stable, with the exception of the fresh fruit and vegetable allergens. In the recent past, it was felt that these heat-labile fresh fruit and vegetable proteins only caused oral allergy syndrome and not anaphylaxis. However, recent reports indicate that up to 30% of OAS reactions can involve anaphylaxis. There are data that point to a heat-stable fraction of these fruit and vegetable proteins that may be responsible for the more severe reactions. As another example, the allergenicity of whey proteins, which are major cow's milk allergens, has been reported to be somewhat susceptible to temperatures of 80-90C. A major egg allergen, ovalbumin, was shown in one study to lose 90% of its allergenicity when treated at 100°C for 3 min. Given these contrasting examples, it is my opinion that heat stability is not a good indicator of potential allergenicity.

Brown Norway Rat Model - There is no validated animal model for food allergy. Among the contenders, though, the model used in this study is one of the farthest from being able to be validated, as it is an injection model, and, as would be expected, registers a response from allergens and non-allergenic proteins alike. A more useful model would be one based on oral gavage or feeding. When and if appropriate animal models become available, Cry9C should be evaluated in those models.

Bioavailability in the Rat - In order for something to become an allergen, various factions of the immune system must be presented with portions of the protein on antigen presenting cells, which necessitates crossing the g.i. barrier via receptors/endocytosis, being actively sampled by M cells, or perhaps via other unknown ways. At the lower dose of 2.6 mg/kg, no Cry9C protein or peptides were found in rat sera, while at higher doses ELISA-reactive protein could be found. This indicates that there is a possibility that at low exposure levels Cry9C may be unavailable to the immune system. The detection limit of the ELISA was 5 ng/ml; it is unknown whether quantities smaller than that were present in the serum. Some types of allergic sensitization can occur at very low levels of allergen exposure, particularly inhalant allergy. In general, it has been shown for food allergy that sensitization requires a fairly high exposure level for an extended period of time. It is my opinion that a low exposure level coupled with the apparent low bioavailability might prevent Cry9C from being allergenic. However, since it is unknown what levels of food allergens cause allergic sensitization, the bioavailability data should be considered in totality with other measurements, and not as a stand-alone entity.

Stability to Digestion - The data on digestion show that no digestion of the Cry9C protein occurs in simulated gastric fluid at pH 2.0; presence or absence of pepsin made no difference at this pH. In the most recent study, in simulated gastric fluid at a more acidic pH of 1.2, degradation of the Cry9C protein was found. In simulated gastric fluid at pH 1.2 and in the presence of pepsin, Cry9C appears to begin to degrade after 30 minutes and appears to be complete at 60 minutes. Studies have shown a range of normal gastric pH; in one study of children 8-14 years old, the mean stomach pH was 1.5; in another study, gastric pH in 66 healthy individuals ranged from 1.0-2.5, and the mean gastric pH in another study of 79 elderly subjects was 1.3. Certainly, many food allergens identified to date are resistant to digestion, but not all. The fresh fruit and vegetable allergens are the best known of the digestion-labile allergens. In addition, we most likely consume tens of thousands of digestion-resistant proteins every day, yet a very few of these are allergens. It is my opinion that the digestion data do not give a prediction of whether or not Cry9C has the potential to be allergenic.

It is my opinion that the data shared by Aventis, taken in total, while not conclusive, provide evidence that of low probability of allergenicity of Cry9C.

Please contact me with any questions on this summary and opinion.

Sincerely,

Susan L. Hefle

Susan L. Hefle, Ph.D.
Assistant Professor and Co-Director
Food Allergy Research and Resource Program
University of Nebraska

APPENDIX 3

WRITTEN STATEMENT BY DR. STEVE L. TAYLOR, FOOD ALLERGY RESEARCH AND RESOURCE PROGRAM, UNIVERSITY OF NEBRASKA

COMMENTS OF STEVE L. TAYLOR, PhD

Professor and Head Department of Food Science and Technology
University of Nebraska - Lincoln

PRESENTED TO THE UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, FIFRA Scientific Advisory Panel on Bt Plant Pesticides: Risk and Benefit Assessments

October 20, 2000 - Arlington, Virginia

I regret that a prior professional commitment in Australia keeps me from attending the science advisory panel in person.[1] As past Chair of an international panel of scientists formed to develop a model approach to assessing the safety of genetically modified foods, I am concerned that the recent incidents with taco shells and unapproved corn may not have been fully understood by the public or the food industry.

First, I must say that I was dismayed that a product was allowed on the market for animal feed use when it had not been approved for human food use. I believe that was a mistake, however I do not believe there has been any risk to the public.

The corn in question, StarLink Bt corn developed by Aventis, is the only product among about 40 genetically modified crops on the market that has not been approved for use in human foods. All biotech crops on the market today have been assessed by the Food and Drug Administration, the Environmental Protection Agency and the Department of Agriculture. The protocols followed by those agencies ensure that any product approved for food use has passed all tests for substantial equivalence and for the safety of the newly introduced gene(s) and proteins and should therefore be considered as safe as its conventional counterpart.

StarLink, was not approved for food use because the product did not pass all screens for allergenicity. The Bt protein in StarLink, Cry9C, does not resemble known allergens, so in fact it may not be an allergen. However, Cry9C was not immediately broken down in digestion tests. Because most food allergens also are not readily digested, EPA wanted more data before concluding that the protein would not become an allergen. On this basis, the agency was correct in awaiting additional information and approval for food use is pending. Other Bt products on the market contain a Cry1 protein, which is digested in a matter of seconds and has passed all of the other screens for allergenicity. Furthermore, Cry1 proteins have been present in foods via Bt sprays used by organic and other farmers for many years.

But was the public at risk because of this incident? I believe it was not.

[1] Dr Taylor's comments will be presented to the SAP on October 20, 2000 by Jason Hlywka, PhD. Dr. Hlywka completed two years of post-doctoral training in a research position with Dr. Taylor at the Food Allergy Research and Resource Program (FARRP) at the University of Nebraska. Dr. Hlywka will be available to respond to questions.

In order for people to become allergic to a protein they must be exposed to it multiple times over an extended period until they become sensitized. The protein must also be present at a relatively high percentage of total protein content. Most allergenic proteins are present at levels of 1 to 40 percent. Aventis indicates that the Cry9C protein is present in corn grain at 0.013 percent, but any taco shells would contain far less due to the presence of other varieties of corn and the use of other ingredients.

It is highly unlikely that Cry9C protein would be present in any corn products at a level of concern. It is important to understand that only a very small amount of StarLink corn was planted, about 350,000 acres among the nearly 80 million corn acres in the United States (0.3 to 0.4 of a percent). That small amount could conceivably be produced by only 100 large farms. Because of the feed-only restriction, nearly all would have been properly channeled to feed operations, but even if the production from some farms was improperly channeled, the amount entering the food supply would be of a relatively low percentage when mingling with other grain is taken into consideration.

This clearly would not produce protein levels of any health concern. It is unfortunate that this incident has sent a negative message to consumers because I believe that U.S. regulatory procedures ensure that any genetically modified crop approved for food use is as safe as its conventional counterpart. StarLink is the only product not approved for food use, and we can almost certainly expect that it will be the last.

Steve L. Taylor, PhD
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APPENDIX 4

CROSS WALK EXPLANATION FOR CORN FRACTIONS IN DEEM™

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CROSS WALK EXPLANATION FOR CORN FRACTIONS IN DEEM™

Many fractions of corn are not final ingredients in human food.

DEEM™ contains the following corn-based ingredient categories:

- Corn, endosperm
- Corn, bran
- Corn sugar (includes high-fructose corn sugar, the solids component of high-fructose corn syrups)
- Corn oil
- Corn sugar/molasses

A key step in the creation of DEEM™ was to prepare a recipe for each food reported to be consumed in the USDA Continuing Survey of Food Intake by Individuals (CSFII). As recipes were prepared, corn products were assigned to one of the five categories listed above. For most items it was a straightforward assignment, e.g. high fructose corn sugar was assigned to corn sugar and corn oil to corn oil. All other items were assigned to the ***corn, endosperm*** category. This included corn starch, grits, corn meal, corn flour, corn gluten, and masa flour.

To date, no hulls, gluten, corn germ, or steepwater has been identified as a component of any CSFII food.

Table 1 lists the quantities of Cry9C protein in processed corn commodities. Animal feed items were not included in DEEM™ recipes unless they were also a human food item. Typical processed fractions that are animal feed items include meal, hull material, composite grits, and gluten.