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Concerning Dietary Exposure To Cry9c Protein Produced By Starlink® Corn And The Potential Risks Associated With Such Exposure

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U. S. ENVIRONMENTAL PROTECTION AGENCY

DRAFT WHITE PAPER

CONCERNING DIETARY EXPOSURE TO CRY9C PROTEIN PRODUCED BY STARLINK® CORN AND THE POTENTIAL RISKS ASSOCIATED WITH SUCH EXPOSURE

Executive Summary

StarLink® refers to a variety of yellow corn genetically engineered to express the protein Cry9C. Cry9C is toxic to various insect pests of corn and acts as a pesticide, therefore its sale and or distribution is subject to regulation by the U.S. Environmental Protection Agency (EPA or Agency) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). Under FIFRA and FFDCA, a company seeking to sell or distribute a pesticide must submit data demonstrating that it will not cause unreasonable adverse effects on the environment and that any residues in food will be safe, i.e., that there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information.

Aventis Agroscience, Inc. (Aventis) submitted data on the safety of StarLink® and applied for approvals under FIFRA and FFDCA. EPA concluded that the available data did not provide enough information to support a conclusion that Cry9C was not a potential human allergen, but that all other information indicated that it would not pose any other types of risks to human health or the environment. Accordingly, in 1998 EPA registered StarLink® for commercial use, provided that all grain derived from StarLink® corn was directed to domestic animal feed or to industrial uses (e.g., biofuels). The intent of requiring all StarLink® to be segregated as either domestic animal feed or for industrial use was to preclude any occurrence of the potentially allergenic Cry9C in human food. The registration contained several specific requirements designed to ensure that no StarLink® grain entered the human food supply. Following registration, relatively small quantities of StarLink® were planted in the United States: 9,018 acres in 1998, 247,694 acres in 1999, and 350,000 acres in 2000, with the largest planting representing less than half a percent of the total acreage planted to corn in the United States.

In September 2000, residues from StarLink® were detected in taco shells, indicating that it had entered the human food supply. In response to these detections, Aventis requested cancellation of the StarLink® registration. In addition, working with U.S. Department of Agriculture (USDA), Food and Drug Administration (FDA), EPA, and the food industry, Aventis undertook a program to remove all StarLink® from the food supply. Among other measures, FDA issued guidance “for sampling and testing

yellow corn and dry-milled yellow corn shipments intended for human food use for Cry9C protein residues" that indicated that "manufacturers who detect Cry9C-containing corn in any lot should divert the lot to animal feed or industrial use".

At the same time, Aventis requested that EPA reconsider its position that the available data did not provide enough information to support a conclusion that Cry9C was not a potential human allergen. Aventis provided additional data and analysis to support its position that the allergenic risks of Cry9C were very small. Most of the arguments advanced by Aventis involved the assertion that exposure to Cry9C was so low, especially after the full implementation of the containment and removal program, that there would be no threat to public health. EPA convened a meeting of its FIFRA Scientific Advisory Panel (SAP or Panel) on November 28, 2000, to consider a series of questions concerning the potential of Cry9C to cause a human allergic response.

Subsequent to the November 2000 SAP meeting, both Aventis and EPA developed additional information and analyses. The Agency convened another meeting of the SAP in July 2001 to review the new information and analyses. Among the materials evaluated by the 2001 Panel was a White Paper developed by EPA that described the corn wet milling process and documented that it removes virtually all of the protein present in corn grain from the various processed food forms produced by wet milling for human consumption - primarily corn syrup, corn oil, alcohol, and corn starch. The SAP commented favorably on this White Paper in which EPA stated that "it is reasonable to conclude that there is virtually no Cry9C protein in wet milled products and that there is no likely health concern for the public associated with the consumption of any food fraction produced by wet milling of corn as long as reasonable steps are taken to ensure that StarLink® corn is not diverted into wet milling."

Following the cancellation of the StarLink® registration, Aventis established a separate corporate entity, StarLink Logistics Inc. (SLLI), as the successor to Aventis' interest in StarLink® products. SLLI oversees the StarLink® Enhanced Stewardship Program, through which SLLI and the U.S. corn millers have continued the efforts to contain and remove Cry9C from the human food supply. SLLI also maintains a monitoring database containing the test results from more than 4 million tests from over 4 billion bushels of corn collected by dry milling facilities and other corn handling operations. These tests were carried out according to guidance developed by FDA and USDA's Grain Inspection, Packers, and Stockyards Administration (GIPSA), and the federal government considers the data reliable.

In 2005, SLLI commissioned Exponent, Inc., to prepare a new exposure assessment of the levels of Cry9C present in the U.S. food supply for submission to EPA. SLLI provided supplemental information in 2006 that updates the 2005 exposure assessment and that quantitatively characterizes the impact of the monitoring and diversion program on exposure to Cry9C. The USDA's Agricultural Research Service (ARS) provided the analytical data on Cry9C concentrations in corn grain used in Exponent's exposure analysis. In addition, the ARS provided results from testing corn seeds from the 1970s and 1980s (that is, before Cry9C was ever bioengineered into corn) for the possible presence of naturally occurring Cry9C or other proteins that give a positive reaction in the Cry9C test. GIPSA conducted additional testing to verify the results of the ARS laboratory.

A careful review of this information shows that the cancellation of the StarLink® registration and the program to contain and remove StarLink® from the corn supply have produced a dramatic decrease in the level of Cry9C estimated to be in the human food supply – from an upper bound estimate of 57 µg/person/day in 2000 to an estimate of high end exposure of 0.007 µg/person/day in 2006.

<u>Conclusion</u>. Based on the following lines of evidence, EPA has concluded that, as of 2006, the potential exposure of the U.S. population to Cry9C in the U.S. food supply is extremely low. Specifically, EPA finds that:

- Levels of exposure to Cry9C estimated in 2006 are dramatically lower than 2000 exposure estimates (~ 8,000 lower at the 99.9th percentile and over 200,000 fold at the 95th percentile).

- A comparison of the estimated exposures to Cry9C from all dietary sources indicates that today they are comparable to or lower than the upper bound estimates of exposure that could result from consumption of corn-based food produced in 2001 by the wet-milling process. For these estimates EPA concluded, based in part on the advice of its SAP, that "there is no likely health concern for the public associated with the consumption of any food fraction produced by wet milling of corn." During the time frame between 2001 and 2007, there have been no reports reliably linking allergic reactions to exposure to Cry9C, suggesting that Cry9C at these levels is not causing allergic responses.

- A probabilistic estimation methodology assessment shows that diversion from the human food supply of corn grain testing positive for Cry9C would have an extremely small effect on reducing the already very low levels of Cry9C in the food supply. That is, very little difference in exposure is observed between: (1) the estimated 2006 exposures that occur when grain that tests positive for the presence of Cry9C is removed from the food supply, and (2) the exposures that could have occurred if such grain had not been tested and diverted but had been allowed to remain in the human food supply.

While each of these lines of evidence has limitations, which are discussed in detail in the White Paper, taken together they strongly support a determination that testing corn grain for Cry9C at dry mills and masa operations is unnecessary since current estimates of potential exposure are such that there is no likely health concern for the public associated with the consumption of corn-based food products, including food products from the dry milled process or masa operations. Therefore, EPA recommends that:

- FDA withdraw its guidance for dry milling facilities and masa operations that recommends sampling and testing yellow corn and dry-milled yellow corn shipments intended for human food use for Cry9C protein residues.

I. Purpose

StarLink® is a variety of corn genetically engineered to express the protein, Cry9C. Because Cry9C was intended to be toxic to various insect pests of corn, Cry9C and the genetic material necessary to produce it are considered a "pesticide" and are regulated by the U.S. Environmental Protection Agency (EPA or Agency) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). Cry9C from StarLink® is slow to degrade in solutions that mimic human digestive fluids, a characteristic that raised concerns about the potential of this protein to elicit allergic responses in humans. Therefore, when EPA approved the sale and distribution of StarLink® in 1998, EPA imposed a series of conditions designed to ensure that all StarLink® grain was directed to channels of trade where it would be used only as domestic animal feed or for industrial purposes. Nonetheless, in 2000 Cry9C was detected in human food. This discovery led to the cancellation of the StarLink® registration and a concerted effort to remove all grain testing positive for Cry9C from the human food supply.

This paper (1) assesses the effect of those efforts on the levels of exposure by comparing the levels of exposure to Cry9C that are occurring today to the levels of exposure estimated to occur in 2000; (2) discusses the significance of these levels of exposure for public health; (3) reviews new information about analytical measurements of Cry9C; and (4) makes public policy recommendations in light of this analysis.

This paper has seven sections. The brief description of the purpose of the paper in this Section is followed by a description in Section II of the legal authority under which EPA regulates pesticides. Section III summarizes the state of the science used to assess the potential allergenic risks to humans posed by the introduction of the StarLink® protein, Cry9C, into the diet. Section IV of the paper presents a full description of the regulatory history of StarLink® corn and particularly emphasizes the various scientific analyses and reviews that were performed to evaluate the potential for risk to public health posed by residues of Cry9C in the food supply. This historical review concludes with the SAP meeting in 2001, the most recent public and external peer review of issues relating to the potential allergenic risk of Cry9C. Section V discusses the new information and analyses developed since 2001, primarily by StarLink Logistics, Inc., (SLLI) and its contractor, Exponent, Inc. Section VI of the White Paper explains how the information and analyses led to EPA's conclusions on the levels of Cry9C currently in the human food supply. Section VII offers EPA's summary recommendation.

II. Legal Framework

The Agency regulates pesticides primarily under two statutes: FIFRA and FFDCA. These laws address the sale, distribution, and use of pesticides (FIFRA) and the safety of pesticide residues in food (FFDCA).

A. FIFRA

StarLink® is the trade name for a variety of corn that has been genetically engineered to produce a protein, Cry9C, that is toxic to certain insect pests of corn. Because the Cry9C protein was intended to prevent, destroy, repel, or mitigate a pest, EPA considered the Cry9C protein and the genetic material necessary for its production, to be a pesticide under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). 7 U.S.C. §§ 136-136y. (When plants have been genetically engineered to express a pesticidal protein, the Agency refers to the pesticide as a "plant-incorporated protectant" or "PIP.") Section 3(a) of FIFRA requires that before a pesticide may be sold or distributed in commerce, it must be registered, or licensed. 7 U.S.C. § 136a(a). Pursuant to Section 3(c)(5) of FIFRA, the Agency may register a pesticide under FIFRA only if EPA concludes, among other things, that use of the pesticide will not cause "unreasonable adverse effects on the environment." 7 U.S.C. § 136a(c)(5)(C). FIFRA defines "unreasonable adverse effects on the environment as ". . . any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide, or a human dietary risk from residues that result from a use of a pesticide in or on any food inconsistent with the standard in section 408 of the Federal Food, Drug, and Cosmetic Act. . . ." 7 U.S.C. § 136(bb). Thus, a pesticide must meet two tests in order to be registered: the benefit of using the pesticide must outweigh its risk, and any residues in food or feed resulting from use of the pesticide must meet the safety standard in FFDCA Section 408.

B. FFDCA

The Agency regulates the safety of any residual amounts of a pesticide or substances resulting from the metabolism or other degradation of a pesticide (collectively referred to as a "pesticide chemical residue") that occur in or on food under the Food, Drug and Cosmetic Act (FFDCA). 21 U.S.C. § 301 et seq. The FFDCA makes it unlawful to sell food – a term that includes food for both humans and animals – if it is "adulterated." Food is adulterated if, among other things, it contains a pesticide chemical residue for which there is no "tolerance" or "exemption from the requirement of a tolerance" or if the food contains a pesticide chemical residue in excess of the level specified in the tolerance applicable to that type of food. Section 408 of the FFDCA provides that EPA "may establish or leave in effect a tolerance for a pesticide chemical residue in or on a food only if the Administrator determines that the tolerance is safe." 21 U.S.C § 346a(b)(2)(A)(i). Pursuant to Section 408, "safe" is defined to mean that "there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." 21 U.S.C. § 346a(b)(2)(A)(ii) or 346a(c)(2)(A)(ii). The FFDCA also allows EPA to establish an exemption from the requirement of a tolerance for a pesticide chemical residue if EPA determines that such residue would be safe. 21 U.S.C. § 346a(c)(2)(A)(i). When EPA establishes an exemption for the requirement of a tolerance for a pesticide chemical residue, a food may contain any amount of that residue. The Agency generally establishes tolerances and exemptions for specific pesticide chemical residues and specific types of food.

III. Scientific Considerations Regarding Allergenicity for the Initial StarLink® Decision

While plant-incorporated protectants generally promise a potentially more benign means of pest control than conventional chemical pesticide alternatives, as part of the risk assessment conducted under FIFRA and FFDCA, EPA must assess whether any protein portion of the PIP could pose an allergenic risk to humans. Beginning with the first field tests of crops genetically engineered to express new traits, the three federal agencies involved in agricultural biotechnology oversight, the United States Department of Agriculture (USDA), the Food and Drug Administration (FDA) and EPA, have sought the most current scientific input on allergenicity through workshops and other public meetings. A 1994 conference in Annapolis, MD on Scientific Issues Related to Potential Allergenicity in Transgenic Food Crops led to the development of an understanding of how sensitization develops into food allergy and the development of a list of features characterizing food allergenic proteins. More recently, the U. S. has participated in the development in the Codex Alimentarius of guidelines[1] for assessing the food safety of foods derived from biotechnology, including the assessment of potential allergenicity of newly expressed proteins in recombinant-DNA plants.

A food allergy is a reaction of the immune system to an otherwise harmless food or food component. Reactions can have a highly variable clinical presentation from as little as mild itching or rash to anaphylactic shock. All allergens are proteins or chemical sensitizers that interact with proteins. In the development of food allergy, a sensitization phase is required where exposure to a substance primes the immune system to react. If sensitization occurs, subsequent exposures to the same substance at sufficient concentrations can then elicit an allergic response. Only a limited number of foods are known to cause the majority of food allergies, and many of the major food proteins responsible for these reactions have been described.

There are no definitive methods to assess potential allergenicity of proteins originating from sources not known to produce food allergy. There are no toxicology tests in the harmonized guidelines used for pesticide registration to evaluate food hypersensitivity reactions. In addition, there is no established animal testing system to evaluate proteins for potential food allergenicity in humans. However, there are some recognized procedures that can be used to evaluate a new protein to assess its allergenic

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[1]Codex Alimentarius *Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants* (CAC/GL 45-2003) available at: http://www.codexalimentarius.net/download/standards/10021/CXG_045e.pdf

potential. These procedures include evaluating the similarity of the new protein to known food allergens with respect to amino acid sequence and examining its biochemical characteristics such as molecular size, resistance to enzymatic and acid degradation, and heat stability. These procedures together form the basis for evaluating whether a given protein is likely to be, or become, an allergen. Even known food allergens do not always share all these characteristics, however, and no one feature is predictive. Therefore, allergenicity assessments, including EPA's, consider a suite of characteristics and use a weight of the evidence approach for the safety determination**.**

Until the application for registering Cry9C in StarLink® from AgrEvo (subsequently merged into Aventis CropScience), all the registered plant-incorporated protectants reviewed by EPA were proteins that had appeared in microbial pesticides, had some previous dietary exposure, and had no similarities or shared biochemical features to known food allergens. While there was no amino acid sequence similarity between Cry9C and known allergens, the company had submitted studies that indicated Cry9C had stability to gastric digestion and heat denaturation and an animal study[2] that suggested the possibility of Cry9C allergenicity. In order to proceed with the registration process, EPA issued an exemption from the requirement of a tolerance for feed uses of StarLink®, incorporated into the registration strict terms and conditions requiring that Aventis take specific measures to ensure that StarLink® corn would be segregated from the human food supply, and prepared for a meeting of its FIFRA Scientific Advisory Panel (SAP or Panel) to address questions about digestive stability and human allergenicity. (The SAP is an advisory committee, chartered under the Federal Advisory Committee Act, composed of independent, external experts in the science of assessing the risks of pesticides.)

IV. History of the Scientific Evaluation and Regulation of StarLink®

A. Registration & Tolerance Exemption for StarLink®

In 1998, EPA received an application to register StarLink® corn under FIFRA and a petition to establish an exemption from the requirement of a tolerance for Cry9C and the genetic material necessary to produce it under the FFDCA from AgrEvo[3]. In 1998, following a thorough scientific review of the safety of StarLink®, EPA concluded that it was unable to determine whether Cry9C would elicit an allergic response in humans. The Agency did determine, however, that aside from this unresolved human allergenicity issue, StarLink® would pose no other risks to public health or the environment, including a finding that there was no risk to humans from eating food from animals that had consumed StarLink® corn.

[2] "Development of new methods for safety evaluation of transgenic food crops" MRID 447140-02. Relevant materials can also be found in the February 29, 2000 SAP meeting at: http://www.epa.gov/scipoly/sap/meetings/2000/february/foodal.pdf

[3] EPA in the July 19, 2001 Federal Register (59 FR 60542) established a tolerance exemption for all DNA and other genetic material that are part of a plant-incorporated protectant. See Title 40 of the Code of Federal Regulations (CFR) at 174.475. However, the AgrEvo petition addressed both an exemption for the residues of Cry9C and the genetic material necessary to produce it as the AgrEvo petition was submitted prior to issuance of the July 19, 2001 Federal Register.

Because StarLink® appeared to offer benefits in controlling important corn pests and the risks of using StarLink® were limited, the Agency concluded that StarLink®'s benefits outweighed the risks of its use – if its use was conditioned to preclude the occurrence of Cry9C in the human food supply. Therefore, in 1998 EPA issued a registration for StarLink® to AgrEvo. EPA limited the registration by requiring that all grain and other products derived from StarLink® corn be used only in domestic animal feed and for industrial purposes. EPA did not approve the use of StarLink® corn in foods destined for human consumption because of unanswered questions about the potential allergenicity of Cry9C. As part of its approval of the registration of StarLink®, the Agency imposed specific terms and conditions on AgrEvo (and its successor, Aventis) that were intended to ensure that the registrant and growers would take appropriate steps to preclude the presence of Cry9C in the human food supply.

Consistent with its regulatory decisions on StarLink® under FIFRA, in 1998 the Agency established an exemption from the requirement of a tolerance for the residues of Cry9C, and the genetic material necessary to produce it, in domestic animal feed (40 CFR 180.1192). Because the exemption did not apply to food products intended for human consumption, the presence of Cry9C from StarLink® in human food products would make such food adulterated.

Aventis began to market StarLink® commercially in 1998, and farmers bought and planted small but increasing amounts of StarLink® corn in 1998, 1999, and 2000. The following table provides information on the amount of StarLink® planted in these years:

Table 1: Corn Acreage Planted to StarLink® by Year

Year	**Acres of StarLink®**	**Percent of U.S. Corn**
1998	9,018	0.01
1999	247,694	0.32
2000	350,420	0.43

The degree to which StarLink® corn penetrated the commercial seed market varied by locality. The highest penetration recorded in any year in any state was 1.5% of the corn planted.

B. February 2000 SAP Meeting

Because of Aventis' continuing interest in obtaining approval for use of StarLink® grain in the production of human food and the novel scientific issues raised concerning the assessment of potential allergenicity, EPA called a meeting of the FIFRA SAP on February 29, 2000, regarding Cry9C. The February 29, 2000, SAP report[4] stated that, with the data available, the SAP could not determine whether or not Cry9C is an

[4] http://www.epa.gov/scipoly/sap/meetings/2000/february/foodal.pdf

allergenic protein. Specifically, "[t]he Panel agreed that based on the available data, there is no evidence to indicate that Cry9C is or is not a potential food allergen." Later the Panel's report stated "The question that must be addressed is what additional data should be collected to assess if Cry9C or any other protein [in] plant-pesticides is a potential food allergen. The level of the allergen in the environment would be useful for its potential as an inhalant or ingestant allergen. Exposure levels are important[.]"

C. 2000 Cancellation of StarLink® and Initiation of Efforts to Remove StarLink® from the Grain and Seed Supply

In September 2000, *cry9c* DNA was detected in a finished food product for human consumption – taco shells. Subsequently, the *cry9c* DNA and Cry9C were found both in corn grain and in other corn products in the human food supply. These detections demonstrated that, despite the EPA restrictions, some quantities of StarLink® had directly entered the human food chain. On September 26, 2000, Aventis discontinued sale of StarLink® for the 2001 growing season and three days later announced an agreement with EPA, FDA, and USDA to locate and contain StarLink® and direct it to approved uses under USDA oversight. The program implementing this agreement is known as the StarLink® Enhanced Stewardship (SES) Program. On October 12, 2000, Aventis requested that the registration for their StarLink® corn product be voluntarily cancelled. Upon EPA's acceptance of the cancellation request in 2000, it became unlawful to sell or distribute StarLink® seed for planting. In addition, USDA, EPA, and FDA worked with the National Corn Growers Association, the American Seed Trade Association, and individual seed companies to institute a testing program to ensure that corn seed for planting in 2001 and future years would be free of Cry9C.

D. November 2000 SAP Meeting

On October 25, 2000, Aventis submitted to EPA an amendment to its pending petition for Cry9C in human food. This amended petition, submitted under the FFDCA, requested a time-limited (four years) tolerance to cover residues of Cry9C that could be present in human food made from StarLink® corn planted in 1998, 1999, and 2000. Aventis submitted a variety of additional information with its petition to support its contention that Cry9C posed no allergenic risk to public health.

EPA convened an SAP meeting on November 28, 2000, to consider a series of questions concerning the potential of Cry9C to be a human allergen, whether there was an amount of the protein in corn sufficient to cause sensitization, and to what levels of Cry9C the public might be exposed if this time-limited tolerance were to be approved. Among other things, EPA prepared a series of "bounding estimates" of the amount of Cry9C exposure possible for different population groups[5]. EPA's estimate indicated that for the general U.S. population, 99.9% of the population would have an exposure lower

[5] Aventis' amended petition also included estimates of potential exposure to Cry9C that were approximately 7 fold lower than EPA estimates. The difference between the EPA and Aventis estimates was due primarily to differences in assumptions about the extent of commingling of StarLink® grain with non-StarLink® grain during grain handling and processing.

than 57 µg/person/day. It should be noted that EPA's assessment indicated that, because of different food consumption patterns, members of some subgroups appeared to receive higher exposure than the general U.S. population, but EPA lacked data adequate to develop reliable estimates of exposure above the 99th percentile of exposure for these subgroups[6]. More information, including the EPA documents submitted for SAP review, other background information, and the SAP final reports can be found on the following web site: http://www.epa.gov/oscpmont/sap/meetings/2000/index.htm#112800.

E. Report from the November 2000 SAP Meeting

The November 28, 2000, SAP concluded that "there is a medium likelihood that the Cry9C protein is a potential allergen based on the biochemical properties of Cry9C protein itself . . . ." (Emphasis in original.) The November 2000 Panel report cited the February 29, 2000 Panel report and found that Cry9C had multiple characteristics associated with known allergens. While noting that these characteristics were not considered "definitive evidence," the Panel concluded that, in light of these factors "[t]aken together, . . . Cry9C protein has a medium probability of being a potential allergen."

At the same time the Panel also concluded that the extent of StarLink® in the human food supply at that time had a "low probability to sensitize some individuals to Cry9C protein." (Emphasis added.) This conclusion rested on three factors: the expression level of Cry9C in corn, the amount of StarLink® corn expected to have entered the human food supply, and the fact that StarLink ®grain was expected to be commingled with grain that did not contain Cry9C. The Panel further stated "lowering the levels of Cry9C in the food supply makes sensitization less probable."

The Panel's November 2000 report also concluded that "the methodology used by the Agency to estimate exposure is defendable." The SAP also noted, however, that the "Agency's analysis results in an upper bound estimate that is considerably high and could justifiably be reduced if [EPA factored several considerations cited by the SAP into its estimate.]" The Panel noted that a more refined estimate of exposure would take into account: (1) a more realistic approach to assessing the extent and impacts of blending StarLink® grain with non-StarLink grain; (2) data from actual monitoring programs to detect StarLink® in grain intended for the human food supply; and (3) the impacts of processing on the likelihood that Cry9C would be present and detected in different, corn-based, processed food products. The Panel indicated that the levels of Cry9C present in such processed foods would vary considerably, depending especially on the method of processing and whether the product was from white or yellow corn. (The *cry9c* DNA was only engineered into certain yellow corn varieties.) The SAP report observed that items

[6] EPA estimated that at several percentiles of exposure the subgroup identified as the general Hispanic population would have exposure to Cry9C higher than the general U. S. population. For example, at the 99th percentile of exposure, the general Hispanic subgroup was estimated to receive approximately 133% of the amount of exposure of U.S. population – 33 µg/person/day vs. 25 µg/person/day.

produced by "wet milling" of corn grain – such as corn syrup, corn oil, and corn starch – contain virtually no protein and therefore would contain virtually no Cry9C[7].

In conclusion, the SAP responded to EPA's question about the overall probability of the risk of significant allergic reactions to Cry9C as follows:

> "The Panel assessed the currently submitted data and concurred, while not conclusive, that the likely levels of Cry9C protein in the U.S. diet of [sic] provide sufficient evidence of a low probability of allergenicity in the exposed population.
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> "This conclusion was based on taking [into] consideration several factors:
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> 1. The moderate allergenicity prediction of the protein.
> 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."

Finally, the Panel observed that "[i]f corn-derived food products contain a highly potent allergen then allergic reactions should appear within a few years."

F. USDA & FDA Guidance Documents on Testing Corn Grain for Cry9C

In response to concern from the milling and food processing industries, during the months following the SAP meeting both the USDA and the FDA developed guidance documents addressing how private entities could reduce the chance that StarLink® grain would be used in the production of human food.

First, in December 2000 the USDA Grain Inspection, Packers, and Stockyards Administration (GIPSA) issued guidance[8] containing a protocol for sampling and testing corn grain to detect the possible presence of Cry9C. This guidance reported that USDA GIPSA had validated two different test kit systems for detecting the presence of Cry9C in corn grain – the TraitCheck Bt9 Lateral Flow Strip (LFS) Test from Strategic Diagnostics, Inc., and the Cry9C QuickStix™ Test Kit from EnviroLogix, Inc. The

[7] EPA did not include corn syrup and corn oil in its dietary assessment presented at the November 28, 2000 SAP meeting because EPA had judged protein to be absent or virtually undetectable in these food products, and therefore that these commodities would not contribute meaningfully to the overall estimate of potential exposure to Cry9C. These materials are available at: http://www.epa.gov/scipoly/sap/meetings/2000/#112800

[8] http://archive.gipsa.usda.gov/rdd/cry9csampling.pdf

GIPSA guidance also recommended using the test kits as part of a specific sampling and testing strategy that would detect the presence of StarLink® grain 99% of the time in a sample that contained more than 0.19% StarLink® grain. This is equivalent to a limit of detection (LOD) for Cry9C in the range of 20 parts per billion (ppb).

Also in December 2000, FDA issued draft guidance[9] to corn dry-milling and masa operations recommending that they perform testing to detect the possible presence of Cry9C in yellow corn and, in certain circumstances, milled yellow corn (i.e., corn meal or corn flour) intended for human food use. The guidance advised corn dry-milling and masa operations to analyze representative samples from every incoming yellow corn shipment. FDA also recommended that corn dry milling and masa operations screen stored inventories of corn or milled corn (meal or flours), if incoming corn shipments or milled corn produced from those shipments had not been screened on arrival. The FDA guidance advised private entities conducting such testing to follow the analytical procedures described in the USDA GIPSA guidance[8]. In the event that a sample tested positive for the presence of Cry9C, the FDA guidance indicated that the lot from which the sample was taken should be diverted to animal feed or industrial use (e.g., the production of ethanol).

G. EPA White Paper on Wet Milling of Corn

Field corn that is made into processed foods for human consumption first undergoes milling. There are two primary types of milling: "dry milling," which primarily produces corn flour and corn meal, and "wet milling," which primarily produces high fructose corn syrup, corn oil, corn starch, some animal feed products, and ethanol. As the November 2000 SAP noted, depending on the food fraction, the wet milling process removes all or nearly all of the protein in corn. In contrast, dry milling processes do not remove protein from products intended for human consumption. (Not surprisingly, all of the food products in which Cry9C had been detected contained dry-milled processing fractions. No samples of food containing only wet milled food fractions have tested positive using the analytical method recommended by FDA and USDA GIPSA.)

In March 2001, EPA issued a White Paper[10] in response to the November 2000 SAP recommendation to examine the impact of wet milling on the levels of Cry9C potentially present in human food made from StarLink® grain. The Agency's White Paper described the wet milling process and documented that it removes virtually all of the protein present in corn grain from the various processed food forms produced for human consumption, primarily corn syrup, corn oil, alcohol, and corn starch. Data showed that corn protein will not be present in corn syrup, corn oil, or alcohol, and that corn starch only contains approximately 0.01% protein. Moreover, Cry9C comprised a very small part of the overall protein content of StarLink® corn grain (approximately 0.13%), and StarLink® represented only a small proportion of the total corn planted in

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[9] The final guidance, issued in January 2001, can be found at: http://www.cfsan.fda.gov/~dms/starguid.html

[10] http://www.epa.gov/oppbppd1/biopesticides/pips/wetmill18.pdf

1998, 1999 or 2000, the only years StarLink® was commercially grown (see Table 1). Therefore, the White Paper concluded that there would be, at most, extremely low amounts of Cry9C in food products made from wet milling of StarLink® corn. As StarLink® was removed from the corn supply, even these low levels would fall until there would be essentially no Cry9C in the processed food for human consumption derived from wet milling.

The 2001 EPA White Paper contained upper bound estimates of the levels of daily exposure to Cry9C that different population groups (the U.S. population, infants, children aged 1-6 years, and children aged 7-12 years) might experience in 2000 as the result of consuming corn starch. Based on this analysis, the highest exposure value estimated was for the general U.S. population in 2000 with 99.5% of the population estimated to have an exposure lower than 0.019 µg/day.

Based on the foregoing analysis, EPA concluded that "it is reasonable to conclude that there is virtually no Cry9C protein in wet milled products and that there is no likely health concern for the public associated with the consumption of any food fraction produced by wet milling of corn as long as reasonable steps are taken to ensure that StarLink® corn is not diverted into wet milling."

H. July 2001 SAP Meeting

The Agency convened another meeting[11] of the SAP on July 17-18, 2001 to review new information and analyses developed subsequent to the November 2000 SAP meeting. The new information included:

- data on the impact of processing and cooking on the levels of Cry9C in various types of finished foods made from corn;
- a description of the Aventis – USDA program to purchase StarLink® corn and to direct such corn to permissible uses;
- a description of the efforts of corn handlers, millers, and food processors to ensure that corn grain was being tested for the possible presence of Cry9C and that quantities testing positive are redirected away from the human food chain;
- data on the presence of antibodies in individuals who reported adverse effects following alleged exposure to StarLink®; and
- reports from the medical community on individuals who claimed to have experienced adverse effects following alleged exposure to StarLink®.

Additional analyses included:

- The 2001 EPA White Paper on the effects of the wet milling process on human exposure to Cry9C; and
- Aventis' revised assessment of exposure to Cry9C in light of the steps taken to prevent further amounts of StarLink® from entering the food supply.

[11] http://www.epa.gov/scipoly/sap/meetings/2001/index.htm#july

The Aventis revised assessment estimated the 99th percentile of exposure to StarLink® derived Cry9C in the diet was 0.39 µg/person/day for the general U.S. population[11]. Consistent with the SAP's advice in its November 2000 report, this revised estimate reflected several improvements: actual measurements of Cry9C in finished foods, other data measuring the effects of processing, inclusion of corn starch as a potential source of protein, and the assumption that all grain containing more than 0.125% StarLink®[12] was being successfully diverted from the human food supply. Aventis characterized its assessment as being conservative, i.e., likely to overestimate potential exposure.

I. July 2001 SAP Meeting Report

The SAP reviewed the materials described above and addressed a series of questions from the Agency that asked for scientific comment on the 2001 EPA White Paper, the Aventis exposure assessment, EPA projections about levels of StarLink® in the corn supply in future years, and the implications of this and other new information on the potential allergenic risks Cry9C might pose in the human food supply.

The SAP commented favorably on EPA's White Paper describing the levels of Cry9C exposure that could result from consumption of food fractions derived from the wet milling of corn. Their report stated that "[t]he Panel concluded EPA used a reasonable approach in determining human dietary exposure from corn starch produced from Cry9C protein containing corn via the wet milling process." The Panel noted that if grain handlers and millers conduct the recommended screening of lots in the corn supply, they should rarely, if ever, process corn containing more than 0.125% StarLink®[12]. If incoming grain is assumed to contain 0.125% StarLink® instead of EPA's assumption of 1.5% StarLink® corn, the estimated exposure to Cry9C protein from consumption of corn starch for the 99.5th percentile of the general U.S. population would be 0.0013 µg/person/day, i.e., approximately 10 fold lower than EPA's initial estimate of 0.019 µg/person/day.

The SAP also commented on the Aventis exposure assessment, which they described as "similar to that used by EPA in calculating exposures to Cry9C protein from corn starch consumption, except for the multiple sources of exposure that substantially complicated the analysis and that carried some important implications for the final result." The Panel questioned the accuracy of the values used by Aventis in the assessment for Cry9C in finished foods. Because Aventis used data from processing studies, as measured by an Enzyme-Linked ImmunoSorbent Assay (ELISA) methodology developed by EnviroLogix, the SAP had concerns that the method might underestimate the amount of Cry9C actually present in the processed food fractions. As a result the Panel concluded "[e]stimated upper bound exposure generated by use of Cry9C protein content of corn intermediates rather than the available finished food data could be several fold greater than the 0.37 µg/day presented in the revised Aventis dietary

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[12] The GIPSA validated test systems are capable of detecting down to ~ 20 ppb Cry9C; this level of detection corresponds to approximately 0.125% StarLink® grain, or 1 in 800 kernels.

exposure assessment." Offsetting this consideration, the Panel noted that the assumption that grain contained 0.125% StarLink® was "highly conservative," in view of the documented effectiveness of the StarLink® containment program. Finally, the Panel noted that there is considerable variability in the amount of corn-derived ingredients used in recipes for the same food, e.g., corn meal used to make corn bread. The Panel suggested such variability could change estimated exposure by as much as 50%.

In response to an EPA question about the levels of Cry9C likely to be detectable in future years, the SAP stated, "The impact of 1998 and 1999 production on Cry9C protein levels in current U.S. grain stocks is small and rapidly diminishing. With continued testing under the GIPSA protocol, redirection of grain testing positive for Cry9C protein, producer control of volunteer corn occurrences, and removal of seed testing positive for Cry9C protein, EPA estimates that Cry9C protein will essentially be gone from corn grain in 2 to 3 years and from finished food products made from such corn in 4 to 5 years. The Panel concurs, although trace amounts of *cry9c* DNA may be detectable far beyond these time frames." (Citation omitted.) In general the Panel complimented the past and ongoing efforts of the government and the private sector to contain StarLink® and recommended that they continue.

The Panel reviewed information from FDA and the Centers for Disease Control (CDC) that followed up on incident reports made by individuals who had experienced physical symptoms that they thought might have been caused by eating foods containing Cry9C. The Panel agreed with the government scientists' assessment that the information was inconclusive, i.e., that it failed to establish any link between exposures to Cry9C and allergic responses. The Panel recommended that the federal government continue to monitor incident reports and investigate any for which such an association appeared possible.

Finally, the Panel addressed the implications of the new information for understanding potential allergenic risks to the U.S. population. The SAP agreed there was no reason to change the earlier conclusion 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 food." The Panel also affirmed the earlier conclusion regarding the probability of an allergenic risk: "the likely levels of Cry9C protein in the U.S. diet provide sufficient evidence of a low probability of allergenicity in the exposed population." While the SAP was aware that new estimates suggested exposure to Cry9C could be lower than previously predicted, the Panel raised several questions about the basis of the new estimates. Specifically, the Panel pointed out possible limitations in the analytical methodology used to measure Cry9C levels in finished food products and the fact that there was no exposure estimate for infants who consume a high corn content diet.

As part of its comments on the potential allergenic risks of Cry9C in the diet, the Panel discussed the ability to estimate a threshold level, below which an allergic response was not expected to occur. The SAP summarized its thinking as follows:

> ". . . the Panel could not determine a threshold level of Cry9C protein where there would be a reasonable scientific certainty that exposure would not be harmful to public health. No reliable data are available on threshold levels of isolated food proteins for inducing allergic response in highly sensitive individuals. Thus, the Panel concluded that based on reasonable scientific certainty, there is no identifiable maximum level of Cry9C protein that can be suggested that would not provoke an allergic response and thus would not be harmful to the public."

J. EPA's July 27, 2001 Note to Correspondents

Concurrently with the release of the July 2001 SAP report, EPA issued a statement in a "Note to Correspondents" in which it characterized several of the report's key conclusions as follows:

- "The Panel also stated that, since there is inadequate information to establish a reasonable scientific certainty that exposure would not be harmful to public heath, they could not recommend establishing a specific tolerance level for StarLink." Accordingly, EPA announced that "establishing a tolerance for StarLink in human food is not currently supported."

- "[T]he Panel endorsed EPA's conclusion that the process of wet-milling corn removes virtually all of the StarLink protein (called Cry9C) from products made for human food. This supports the Agency's determination that there is no public health risk from eating products manufactured from StarLink corn through the wet-milling process, provided that corn utilized in the wet-milling process does not contain significant levels of StarLink."

- "The SAP agreed with EPA estimates that the Cry9C protein in domestic corn supply will decline rapidly after the 2001 crop is harvested and with each subsequent production year."

V. Information Generated Since 2001 on StarLink®

Since the 2001 SAP Meeting, EPA has received several studies and analyses addressing Cry9C. These studies and analyses include: results from the SLLI Quality Check Program (QCP)[13], assessments of dietary exposure to Cry9C by Exponent, Inc., (a contractor of SLLI) in 2005 and 2006, a report from USDA's Agricultural Research Service (ARS) Market Quality and Handling Research laboratory in Raleigh, NC on

[13] Following the cancellation of the StarLink® registration, Aventis established a separate corporate entity, StarLink Logistics Inc. (SLLI), as the successor to Aventis' interest in StarLink® products. SLLI oversees the StarLink® Enhanced Stewardship Program, including the Quality Check Program (QCP), through which SLLI and the U.S. corn millers have continued the efforts to contain and remove Cry9C from the human food supply. SLLI also maintains a monitoring database containing the test results from more than 4 million tests from over 4 billion bushels of corn collected by dry milling facilities and other corn handling operations. These tests were carried out according to guidance developed by FDA and USDA GIPSA.

testing corn grain samples pre-dating StarLink®, a 2007 report from a GIPSA Technical Services Division laboratory on retesting of selected samples, and a "tumbled sample" experiment performed in 2007 by the GIPSA laboratory.

A. Ongoing Results of the SLLI Quality Check Program (QCP)

In October 2000 Aventis initiated the StarLink Enhanced Stewardship Program to purchase corn that was determined to contain StarLink® grain. Once purchased, Aventis arranged for the grain to go to domestic animal feed or industrial uses[13]. Since 2000 and continuing to the present, Aventis and its successor entity, SLLI, have provided LFS testing kits[14] to dry milling facilities and other grain handling operations so that they could, as recommended by FDA, conduct testing on shipments of corn to detect the possible presence of Cry9C. Over 400 facilities have participated and have conducted over 4 million tests on over 4 billion bushels of grain.

The results represent the most comprehensive database on the levels of any substance in the food supply. The database shows detection of Cry9C in a shipment of corn grain becoming an increasingly rare event as measured by the LFS test methodology: in the testing period from September 2004 to August 2005, 99.96% of all grain sampled tested negative[15], and in the testing period from October 2005 to September 2006, 99.99% of all grain sampled tested negative[16]. In contrast, in April and August 2001 only ~ 90.5% of the grain tested was negative.[17] From October 2005 through September 2006, positives represented approximately 0.01% of the approximately 70,000 tests using the LFS test conducted each month[16]. This means that the percentage of samples testing positive for Cry9C has declined 950 fold since 2001. The results of testing collected by SLLI from January 2000 through September 2005 are depicted graphically in Figure 1 (from Exponent 2005 report). The data obtained since September 2005 show further declines in detection frequency.

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[14] The LFS test is an immunoassay test based on antibodies specifically directed at the protein Cry9C. It does not quantify the amount of protein present, but rather indicates the presence of a substance that reacts with the antibodies. The LFS test has a limit of detection (LOD) of ~ 20ppb. The LFS test is designed to be a quick immunoassay that can be used in the field.

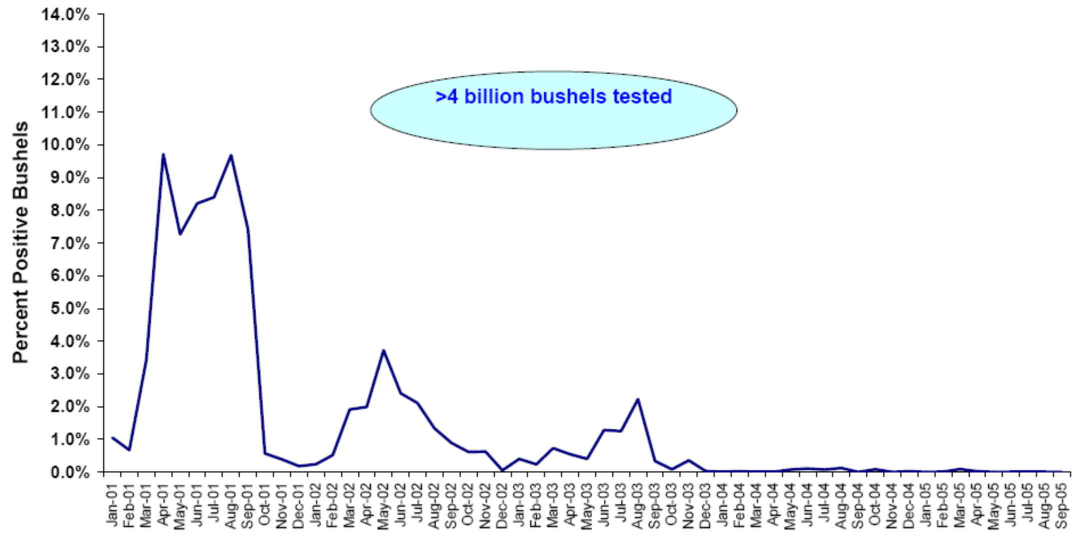
[15] Exponent November 16, 2005 Report. "Assessing exposure to Cry9C protein in StarLink corn".

[16] Exponent December 6, 2006 External Memorandum. "Revised StarLink corn risk assessment and updated QCP data through October 2006".

[17] Exponent November 16, 2005 report. "Assessing exposure to Cry9C protein in StarLink corn." Independent testing in 2001 by USDA reported a high value of ~ 14% of grain testing positive (86% negative) for Cry9C (as reported in Exponent 2005 report).

FIGURE 1

PERCENT OF THE TOTAL BUSHELS OF CORN SAMPLED IN THE QUALITY CHECK PROGRAM SCREENING POSITIVE USING THE STRIP TEST FOR CRY9C FROM JANUARY 2001 THROUGH SEPTEMBER 2005



In addition, individual seed companies producing seed corn for planting in years after the cancellation of StarLink® have reported to EPA that they have conducted testing of their seed lines to ensure they were free of Cry9C. While the Agency does not have access to the results of the seed companies' tests, it seems apparent from the overall low frequency of detection of Cry9C in grain in recent years that new corn seed planted after 2000 contains at most minute amounts, and, possibly, no StarLink® derived Cry9C.

B. Exponent's 2005 and 2006 Assessments of Dietary Exposure to Cry9C

In November 2005, Exponent, a contractor for SLLI, prepared an analysis[15] estimating the levels of exposure to Cry9C in the U.S. food supply. The Exponent 2005 assessment estimated consumer exposure by utilizing the results of monitoring of Cry9C residues in the US corn supply by government and industry using the LFS test on samples collected "in market" as corn is stored, shipped, and/or milled; additional confirmation and quantification studies performed by the USDA ARS using the ELISA plate test; and food consumption surveys for the U.S. population. EPA, USDA, and FDA have reviewed the Exponent analysis and conclude that it provides a reliable basis for characterizing the distribution of potential exposure to Cry9C for the general U.S. population and significant subgroups during 2005.

The Exponent analysis presents a distribution of potential exposures based on a probabilistic estimation methodology that takes into account the fact that any individual's exposure depends on two independent factors: (1) how much Cry9C (if any) is present in food derived from yellow corn, and (2) how much food derived from yellow corn the person consumes. The following paragraphs provide a brief description of Exponent's exposure analysis.

As the first step in estimating levels of Cry9C in food, Exponent created a representative distribution of Cry9C in the corn grain supply based on:

(1) The QCP database, a compilation of the results of extensive screening for the presence of Cry9C in the human grain supply, provided some three hundred samples testing positive for Cry9C in the field from March 2004 to September 2005: The USDA ARS laboratory in Raleigh, NC reanalyzed samples in order to confirm and quantify the amount of Cry9C using both the LFS test (LOD ~ 20 ppb) and the more sensitive ELISA plate test (detection threshold = 0.0655 optical density units, corresponding to about 0.1 ppb); and

(2) The Federal Grain Inspection Service (FGIS) of USDA GIPSA collected more than a thousand one hundred corn samples from mid-April through mid-August 2005 from a variety of locations throughout the corn industry: The USDA ARS laboratory in Raleigh, NC analyzed these samples using both the LFS test (LOD ~ 20 ppb) and the more sensitive ELISA plate test (detection threshold = 0.0655 optical density units, corresponding to about 0.1 ppb).

Exponent then divided the resulting data into six categories ("distributions" in the Exponent report) as follows[18]:

Table 2: Description of "Distributions" for Exponent's Exposure Assessment

Distribution	Field Test Using Lateral Flow Strip	Laboratory Test Using Lateral Flow Strip	Laboratory Test Using ELISA Plate Test
1	n.a.*	–	–
2	n.a.*	–	+
3	+	–	–
4	+	–	+
5	+	+	–
6	+	+	+

*Not available but assumed to be negative for purposes of Exponent's analysis.

Corn in distribution 1 is assumed to test negative in the field for Cry9C using the LFS test, would test negative in the laboratory when retested for Cry9C using the LFS test and the more sensitive ELISA plate test.

Corn in distribution 2 is assumed to test negative in the field for Cry9C using the LFS test, would test negative with the LFS test in the laboratory but positive for Cry9C when retested in the laboratory using the ELISA plate test.

[18] None of the samples that tested negative with the LFS test in the field tested positive in an LFS test performed in the laboratory. Thus, there are only six distributions rather than eight.

Corn in distribution 3 would test positive in the field for Cry9C using the LFS test, negative for Cry9C when retested in the laboratory using the LFS test and negative for Cry9C when retested in the laboratory using the ELISA plate test.

Corn in distribution 4 would test positive in the field for Cry9C using the LFS test method, negative when retested in the laboratory using the LFS test but positive for Cry9C when retested in the laboratory using the ELISA plate test.

Corn in distribution 5 would test positive in the field for Cry9C using the LFS test, positive for Cry9C when retested in the laboratory using the LFS test, but negative for Cry9C when retested in the laboratory using the ELISA plate test.

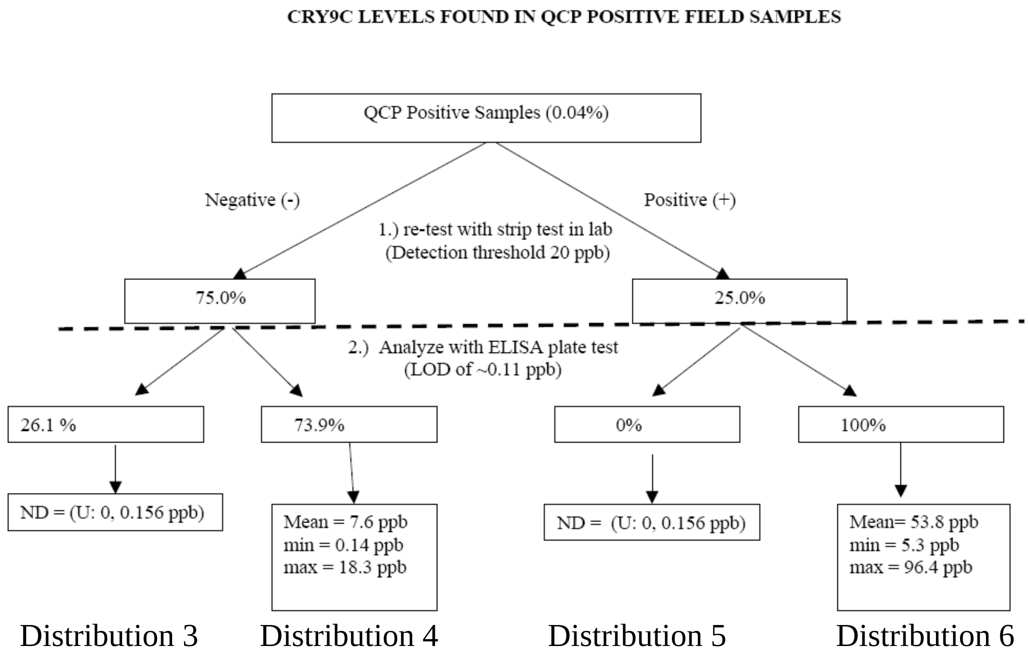
Corn in distribution 6 would test positive in the field for Cry9C using the LFS test, positive when rested in the laboratory using the LFS test, and positive for Cry9C when retested in the laboratory using the ELISA plate test.

The likelihood grain would produce a positive in a LFS test under field conditions was based on the sampling results from the QCP for the time period, September 2004 through August 2005[19]. In order to confirm positive results, samples testing positive in the QCP were sent for laboratory analysis at the USDA ARS laboratory in Raleigh, NC. These samples underwent both a new round of the LFS test under laboratory controlled conditions, as well as an ELISA plate test. These testings allowed for the identification of any false positives in the field testing and quantification of the amount of Cry9C present in positive samples, since the LFS test method only provides a qualitative answer regarding the presence of Cry9C at or above the LOD of ~ 20 ppb[20]. The results of these tests were used, as shown in Figure 2, to characterize the levels of Cry9C in "distributions" 3 through 6.

[19] Two types of locations test for StarLink® corn within the QCP – mills and elevators (including export terminals). Elevator data are classified into corn tested inbound to the elevator and corn tested outbound from the elevator. According to the 2005 Exponent report, in the QCP 0.020% of yellow corn tested positive for Cry9C using the LFS test. Of the corn tested at elevators, 0.024% of corn tested positive (0.019% inbound, 0.028% outbound). Of the corn tested at mills, 0% tested positive. Since the same corn may have been tested at multiple locations, Exponent attempted to allow for this by depending on results from a single site in its assessment, i.e., at elevators. Results from testing conducted on outbound corn samples at elevators were used as an estimate of the prevalence of Cry9C in the U.S. corn supply. As a further conservative measure, all positive results from corn testing positive inbound to elevators was added to the positive results of corn testing positive outbound from elevators, but the denominator was not changed. This resulted in the estimate of percentage positive increasing to 0.04%. The mill results in the QCP were not included since mill testing historically shows a lower prevalence of positive results; most likely because the corn has previously been screened at the elevator and lots testing positive diverted.

[20] The ELISA plate test, also used in assessing the amount of Cry9C in the corn supply, is based on antibodies specifically directed against Cry9C protein. The ELISA plate test, however, can be used to quantify the amount of Cry9C present in a sample. It generally has lower limits of detection than the LFS test. Current laboratory results indicate that the average LOD is approximately 0.115 ppb but can range from 0.076 to 0.156 ppb.

Figure 2 (from 2005 Exponent Report): Derivation of Exponent's Distributions 3-6*

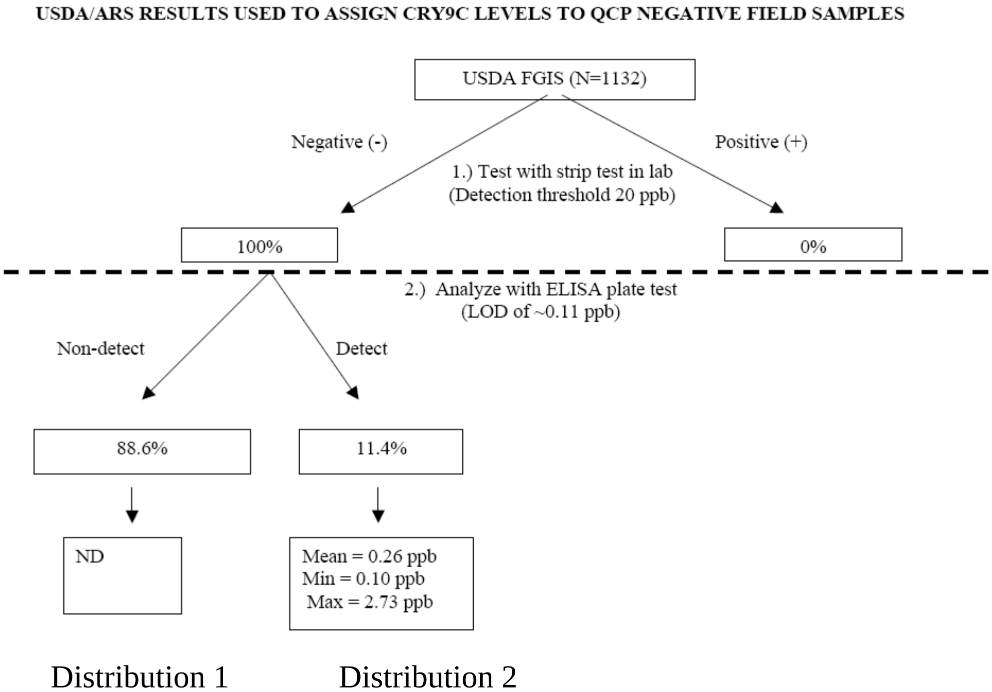


*QCP = Quality Check Program; LOD = Limit of Detection; ND = non-detect; U indicates that the Exponent assessment assumed Cry9C residues were uniformly distributed between the indicated upper and lower limits.

The second source of samples was the USDA GIPSA Federal Grain Inspection Service (FGIS). FGIS randomly collected, in the period from mid-April to mid-August 2005, over 1100 samples of corn grain from throughout the U.S. These samples were shipped to the USDA ARS laboratory at Raleigh, NC. ARS analyzed them in the laboratory using the LFS test and the more sensitive ELISA plate test to characterize the levels of Cry9C in grain. Samples were collected from locations and at a time of the year that would enhance finding Cry9C if it were present in the market. All samples tested negative by the LFS test. For these ELISA plate tests conducted by ARS, the LOD for Cry9C was ~ 0.11 ppb. The amounts detected ranged from the LOD up to 2.73 ppb[21]. These results were used, as shown in Figure 3, to characterize the levels of Cry9C in "distributions" 1 and 2.

[21] It is worth noting that all of the quantified positive detections in this USDA survey correspond to amounts of residue that represent substantially less than would be contributed by a single kernel of corn in the sample of 800 kernels. Since the level of Cry9C in a kernel of StarLink® corn is approximately 13,000 ppb, a single kernel of StarLink® among 800 kernels of corn would correspond to ~ 20 ppb.

Figure 3 (from 2005 Exponent Report): Derivation of Exponent's Distributions 1-2*



*FGIS = USDA Federal Grain Inspection Service; LOD = Limit of Detection; ND = non-detect.

The estimated proportion of the corn grain supply for each distribution is shown in Table 3. For each of the distributions, Exponent assigned a residue value or range of values to reflect the amount of Cry9C likely to be present in grain derived from that distribution.

Table 3: Proportion of Corn Supply Assigned by Exponent to Distributions and Residue Values of Cry9C in 2005 Exponent Assessment

Distribution	**% Occurrence in Corn Supply**	**Residue value**
1	88.569% [= 99.96% x 88.6%]	All values = 0 ppb
2	11.391% [= 99.96% x 11.4%]	Values = empirically observed values in 2005 testing Mean = 0.26 ppb, Min = 0.10 ppb, Max = 2.73 ppb
3	0.008% [= 0.04% x 75% x 26.1%]	Values = a uniform distribution from 0 ppb to the LOD for the ELISA plate test, where LOD ranges from 0 to 0.156 ppb
4	0.022% [= 0.04% x 75% x 73.9%]	Values = empirically observed values in 2005 testing Mean = 7.6 ppb, Min = 0.14 ppb, Max = 18.3 ppb
5	0.000% [= 0.04% x 25% x 0%]	No samples fell into this distribution (see Figure 2); all samples testing positive in the field and laboratory LFS tests also tested positive in the ELISA test and were assigned to distribution 6.
6	0.010% [= 0.04% x 25% x 100%]	Values = empirically observed values in 2004-05 monitoring database (Mean = 53.8 ppb, Min = 5.3 ppb, Max = 96.4 ppb), supplemented by an exponential "tail" (Because of the limited number of data points in the database, Exponent supplemented the empirical values to address the possibility that the food supply potentially contained some grain with higher levels of Cry9C than the maximum (96 ppb) seen.)

Exponent then estimated the levels of Cry9C in food products commonly consumed by humans (e.g., hush puppies, corn bread, polenta, tortillas, taco shells, corn meal and flour) by adjusting the values obtained above with data showing how the level of Cry9C potentially present in the raw corn grain would be affected by the processing used to produce the food[22]. These processes include the "masa" processing and the blending, grinding, intense cooking, and extruding processes used to produce corn-based cereals.

[22] See Table 6 of the Exponent 2005 report which is based on the study submitted by Aventis (MRID 453866-03) and reviewed by the SAP at the July 17-18, 2001 meeting. "Estimate dietary intake of Cry9C protein based on measurements of Cry9C in process foods made from 100% StarLink™ corn."

To estimate consumption of corn-derived food, Exponent used a USDA database on food consumption in the United States, the Continuing Survey of Food Intakes by Individuals (CSFII), and a second database developed jointly by EPA and USDA, the Food Consumption Intake Database, that contains recipes for calculating the amounts and forms of constituent agricultural commodities (corn oil, corn meal, sugar, etc.) that are present in various types of finished foods (e.g., cornbread), which people report in the CSFII having eaten. Exponent's approach to estimation of food consumption of corn-derived food follows standard EPA estimation methodology.

Exponent used the data described above to develop a probabilistic exposure assessment for dietary exposure to Cry9C. A probabilistic exposure assessment is designed to capture the variability in exposure that can result from the fact that each person's daily intake of Cry9C will depend on multiple factors: which foods the person eats, how much of the specific foods he or she eats, and how much (if any) Cry9C is present in the different foods. Because there are a huge number of possible combinations of foods and residues, the variability is assessed using a computer program that randomly combines different diets with different residue levels. The computer program performs tens of thousands of estimates combining the randomly selected values to generate a large distribution of potential exposure values. Because multiple individual exposures comprise the distribution, it is possible to estimate the specific level of exposure received by a particular percentile of the population. The results of Exponent's assessment are shown in Table 4.

Table 4: Exponent's 2005 Dietary Intake Estimates of Cry9C Protein (µg/person/day)

Population Group	**Mean**	**95th Percentile**	**99.5th Percentile**	**99.9th Percentile**
U.S. Population	0.00007	0.00005	0.00303	0.00821
U.S. Children, 1 – 6	0.00005	0.00004	0.00210	0.00577
U.S. Children, 7 – 12	0.00006	0.00007	0.00246	0.00677
Hispanic Population	0.00006	0.00006	0.00233	0.00642

Exponent's assessment uses a number of assumptions that can affect the resulting exposure estimates. To illustrate the effect different assumptions might have on the exposure estimate, Exponent performed four analyses, substituting one alternative assumption in each while holding the other assumptions constant:

Assumption substitution 1. As noted in Table 3, Exponent assigned a uniform or empirical distribution for each of the "distributions" except for distribution 6 for which they used both empirical data and an exponential tail. Exponent conducted a sensitivity analysis making an alternative assumption, i.e., that all of the observed residue values in distributions 2, 4, and 6 were part of a single, lognormal distribution. Rather than assuming the residue values were as described in Table 3, Exponent used modeling techniques to generate the best estimate of a single, lognormal distribution for Cry9C residues. Fitting the data to a lognormal distribution would likely overestimate the amount of Cry9C in the food supply. Using this assumption and keeping other assumptions constant, Exponent estimated exposures as seen in Table 5.

Table 5: Exponent's 2005 Dietary Intake Estimates of Cry9C (µg/person/day), Assuming Residues are Lognormally Distributed[23]

Population Group	**Mean**	**95th Percentile**	**99.5th Percentile**	**99.9th Percentile**
U.S. Population	0.000178	0.000269	0.0070821	0.023896

The values in Table 5 are 2.5 fold (mean); 5.4 fold (95th percentile); 2.3 fold (99.5th percentile); and 2.9 fold (99.9th percentile) higher than the estimates reported in Table 4 for the corresponding percentiles. A comparison of the exposures estimated for the other population subgroups shows a similar range of differences.

Assumption substitution 2. In the original assessment, it was assumed that there were no detectable residues of Cry9C in cereals due to the extensive blending and mixing involved in their preparation. Exponent performed a sensitivity analysis assuming that some residual level of Cry9C could be found in processed cereals. For this analysis, Exponent assigned a Cry9C level of 0.036 ppb to all cereals. This level was determined as the weighted average of the Cry9C levels from the six distributions in the 2005 report. All other assumptions were kept constant. Exponent exposure estimates under this scenario are presented in Table 6.

Table 6. Exponent's 2005 Dietary Intake Estimates of Cry9C (µg/person/day), Assuming Some Residual Level of Cry9C Would be Found in Cereals[23]

Population Group	**Mean**	**95th Percentile**	**99.5th Percentile**	**99.9th Percentile**
U.S. Population	0.000205	0.000999	0.003386	0.008526

Assumption substitution 3. Extensive mixing of corn occurs as it moves from the farm to elevators to mills and finally to food processors. Through storage, tempering, multiple grinding/sifting operations, transfer to storage bins, further processing into retail products, there are at least 7 to 8 distinct points of dilution during the entire voyage from field to end-user. An estimate of the commingling /dilution factor for grain at one specific dilution point, e.g., in an elevator, is on the order of 3 to 5 times, while dilution at the mill is probably much greater. In the Exponent estimate leading to Table 4, only a single occasion of commingling, with 5 fold dilution, was incorporated into the assessment. The process of commingling and the potential for "hot spots" was addressed by dividing the distribution of Cry9C levels into 10 strata representing the 10 deciles of the

[23] Estimates for U.S. children 1 to 6 years of age, U.S. children 7 to 12 years of age, and for Hispanic populations can be found in the 2005 Exponent report.

distribution before adjustment for dilution and commingling. This approach is a worst case estimate in that it forms composite samples within strata that have similar levels, and thus is not likely to form composite samples from corn with low Cry9C levels with corn with high Cry9C levels. Exponent performed a sensitivity analysis assuming no commingling; such an assumption is likely to result in an overestimate of exposure. Keeping all other assumptions constant, the exposure estimates resulting from this analysis are presented in Table 7.

Table 7. Exponent's 2005 Dietary Intake Estimates of Cry9C (µg/person/day), Assuming No Commingling of Grain[23]

Population Group	**Mean**	**95th Percentile**	**99.5th Percentile**	**99.9th Percentile**
U.S. Population	0.000069	0.00003	0.002677	0.008257

Assumption substitution 4. In the original assessment, corn testing negative with the LFS test in the field and subsequently testing negative with the ELISA plate test was assigned a 0 ppb Cry9C level (distribution 1). Exponent performed a sensitivity analysis in which distribution 1 was assigned a uniform distribution ranging from 0 ppb to 0.156 ppb, the highest LOD seen with the ELISA plate test. All other assumptions were kept constant. The exposure estimates resulting from this analysis are presented in Table 8.

Table 8. Exponent's 2005 Dietary Intake Estimates of Cry9C (µg/person/day), Assuming a Uniform Distribution in Distribution 1 of Cry9C Ranging from 0 ppb to 0.156 ppb[23]

Population Group	**Mean**	**95th Percentile**	**99.5th Percentile**	**99.9th Percentile**
U.S. Population	0.000207	0.00106	0.004543	0.009298

As can be seen from Tables 5 through 8, all these analyses produced exposure estimates at or below the upper bound exposure estimate from wet-milled corn products alone (i.e., less than 0.01959 µg/person/day).

In EPA's view, most of the other assumptions in Exponent's assessment tend either to overstate somewhat or to have no effect on the estimate of potential exposure. The assumptions and the manner in which they tend to bias Exponent's exposure assessment are discussed in Table 9.

Table 9: Effect of Key Assumptions on Exponent's 2005 Exposure Assessment

Exponent Assumption	**Effect of Assumption**
Different assumptions regarding the choice of residue values for each distribution (see Table 3).	Compared to an assumption that all residues are part of a single, log normal distribution, understates potential exposure by < 3 fold at the highest percentiles.
All grain testing positive for Cry9C remains in the human food supply.	If anything, slightly overstates potential exposure.
The results of the ELISA assay at low levels reflect levels of Cry9C produced by StarLink®.	If some residues in corn grain detected in the 2005 USDA FGIS survey (distribution 2) are not attributable to Cry9C from StarLink®, the Exponent estimates would overstate the potential exposure to Cry9C.
Commingling of grain occurs at a single point and results in a mixing of each shipment with 5 other shipments of comparable size.	An assumption of no commingling yields similar exposure assessments. Assuming greater rates of commingling would likely yield somewhat lower estimates of exposure at higher percentiles.
Heat processing (e.g., cooking) does not reduce levels of Cry9C.	Overstates exposure to the extent heat processing destroys Cry9C.
Processing of breakfast cereal destroys all Cry9C.	Assuming breakfast cereals retain low levels of Cry9C yields similar exposure estimate.
Samples assigned to distribution 1 contain 0 ppb Cry9C.	If the distribution of residues in distribution 1 samples was assumed to be uniform and to range between 0 and the LOD of the ELISA plate test, the mean would be three fold higher and 99.9th percentile 10% higher (0.008 vs. 0.009 µg/person).

In December 2006[16], Exponent updated its estimates of exposure to Cry9C in the diet, relying on data collected from October 2005 through September 2006 from the Quality Check Program. These data showed the frequency of positive test results using the LFS test on samples of grain in the corn transport and processing system declined from 0.04% to 0.01%. Based on these data, Exponent adjusted the percentages in Table 3 in the various distributions to reflect the less frequent detection of Cry9C in field LFS tests, and using the same assumptions and methodology as it employed in the 2005 assessment, estimated dietary exposure. The results of the updated assessment appear in Table 10. A comparison of Table 10 with Table 4 shows a decrease in dietary exposure in all percentiles of all population groups.

Table 10: Exponent's 2006 Dietary Intake Estimates of Cry9C (µg/person/day)

Population Group	**Mean**	**95th Percentile**	**99.5th Percentile**	**99.9th Percentile**
U.S. Population	0.00006	0.00005	0.00288	0.00747
U.S. Children, 1 – 6	0.00004	0.00004	0.00199	0.00535
U.S. Children, 7 – 12	0.00005	0.00007	0.00234	0.00597
Hispanic Population	0.00004	0.00006	0.00217	0.00589

C. Exponent's 2006 Analysis of the Influence on Human Dietary Exposure of Continued Monitoring and Diversion of Lots Testing Positive for Cry9C

At EPA's suggestion, in 2006 Exponent performed additional analysis to assess the effect on exposure of the ongoing program to test corn grain for the presence of Cry9C in order to divert all lots testing positive to domestic animal feed or industrial uses. Exponent executed this assessment by evaluating the effect on the exposure estimates of omitting from the analysis all samples testing positive in the field. Table 11 incorporates the estimates resulting from this analysis with the 2006 exposure estimates of Table 10. Because Table 10 estimates the amount of Cry9C that may be present in the corn transport and processing system prior to diversion[19], this comparison essentially offers an estimate of the effect of diversion on human dietary exposure. The comparison shows that estimated exposures should not be substantially increased if testing and diversion were to be terminated. For example, for the 99.9th percentile of the U.S. population, Exponent's analysis estimates exposure could increase by 0.000185 µg/person/day if testing and diversion ceased.

Table 11: Comparison of Exponent's 2006 Dietary Intake Estimates of Cry9C Assuming Lots Testing Positive are Diverted with Exponent's 2006 Dietary Intake Estimates of Cry9C Assuming Lots Testing Positive Remain in the Food Supply (µg/person/day)

Population Group	**Mean**	**95th Percentile**	**99.5th Percentile**	**99.9th Percentile**
U.S. Population (Without diversion)	0.00006	0.00005	0.00288	0.00747
U.S. Population (With diversion)	0.000054	0.000048	0.002863	0.007285
U.S. Children, 1 – 6 (Without diversion)	0.00004	0.00004	0.00199	0.00535
U.S. Children, 1 – 6 (With diversion)	0.000037	0.000034	0.001936	0.005135
U.S. Children, 7 – 12 (Without diversion)	0.00005	0.00007	0.00234	0.00597
U.S. Children, 7 – 12 (With diversion)	0.000047	0.000066	0.002296	0.005685
Hispanic Population (Without diversion)	0.00004	0.00006	0.00217	0.00589
Hispanic Population (With diversion)	0.000042	0.000057	0.002167	0.005955

D. Results of Monitoring for StarLink® Since August 2005

The data from SLLI's ongoing Quality Check Program confirm that levels of Cry9C in corn grain continue to diminish. As noted above, the percentage of grain testing positive using the analytical method recommended by FDA and GIPSA (three 800 kernel samples tested using the LFS test) in the 12 month period from October 2005 through September 2006 is only 0.01%, compared to 0.04% positive grain for the period September 2004 through August 2005. In addition, GIPSA continues to fulfill requests to analyze corn grain for the presence of StarLink® using the analytical method recommended by FDA and GIPSA and reports the results of its testing data monthly. GIPSA has not had a positive test since April 2005[24]. Like the QCP program, these results indicate that detection of Cry9C in samples is increasingly rare.

E. Results of Testing Corn Grain Samples Pre-Dating StarLink®

In addition to the results discussed above, SLLI supplied EPA with the results of testing performed by the USDA's ARS Laboratory in Raleigh, NC. ARS analyzed several samples of corn seed for the possible presence of Cry9C using the ELISA plate

[24] GIPSA June 26, 2007 StarLink Test Results.

test, EnviroLogix™ QuantiPlate™ Kit for Cry9C High Sensitivity Protocol[25]. ARS tested 31 breeding lines of corn developed in the 1970s and early 1980s, well before research and development began on StarLink® or any other varieties of genetically engineered corn, and stored from that time by a corn breeder. For each of the 31 lines, five seeds were taken and tested by ARS (total of 155 kernels). Two separate aliquots were analyzed from each single kernel sample. Of the 155 kernels, 135 single kernels registered an optical density above the background level, but only 16 single kernels (~ 10%) produced results greater than the LOD of ~ 0.52 ppb in at least one of the aliquots. Of these 16, there was only one sample in which both aliquots produced results greater than the limit of detection (1.07 ppb and 1.19 ppb). When the results of the two aliquots were averaged for each of the 16 seed samples, the results suggested Cry9C levels up to 5.22 ppb, with 14 of the seeds containing less than 2 ppb. However, in most cases, the two aliquots gave highly variable (and, therefore, suspect) results. For example, one seed produced 10.11 ppb in one aliquot and 0.33 ppb in another. Other examples of variable aliquot pairs include: 2.99 and 0.14 ppb; 5.03 and 0.16 ppb; 1.45 and 0.02 ppb.

Neither ARS nor EPA can definitively explain these results. Since the corn seeds were grown before StarLink® was first created, it seems impossible that an ELISA plate test is measuring Cry9C produced by StarLink®. EPA has considered three other possible explanations. First, there is some suggestion that variation among reagents and materials is affecting the results. It is also possible that at this level of sensitivity, the ELISA plate test is detecting the presence in corn samples of other substances that react with Cry9C antibodies in the ELISA plate test, yielding apparently positive results. Third, these old samples of corn could contain trace background levels of naturally occurring Cry9C because some strains of the natural bacterium *Bacillus thuringiensis,* which are natural components of plant surfaces, have the gene for Cry9C and can express this protein in certain circumstances. Neither the ELISA plate test nor the LFS test is able to distinguish between StarLink® Cry9C protein and Cry9C protein from other sources. Any of these possibilities could result in a “false positive” reading for Cry9C in corn seed grown before StarLink® was created.

In an attempt to better understand these results, EPA asked GIPSA to retest seeds from the pre-StarLink seed lots that generated positive ELISA plate test results in the ARS laboratory. Using the same Envirologix high sensitivity ELISA protocol with an empirically determined LOD lower than that of the ARS laboratory, GIPSA[26] was unable to verify the earlier findings of the ARS laboratory on the seeds. None of the seeds GIPSA tested were positive for Cry9C above the estimated LOD[27].

At this point, it is uncertain why GIPSA was unable to verify the ARS testing data for pre-StarLink corn seeds. Possible explanations include the following:

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[25] Whitaker/Slate November 14, 2006 Memorandum. “ELISA plate test results for Cry9C in corn grown prior to 1990”.

[26] GIPSA June 20, 2007 Analysis and July 12, 2007 Addendum. “Analysis of individual corn kernels using ELISA-based technology to detect Cry9C in StarLink®”.

[27] GIPSA, however, did confirm a positive result for the one sample where ARS was able to provide to GIPSA test material that ARS had extracted and tested (described in the November 14, 2006 memorandum) and found positive.[Personal communication GIPSA].

- Since both ARS and GIPSA conducted tests on individual seeds, ARS may have tested seeds that could give a positive ELISA reading while GIPSA tested seeds that did not contain material that could give a positive reading (sample variability).
- The ARS laboratory may have been using faulty reagents (e.g., poor quality ELISA plates) or had a source of sample contamination (e.g., StarLink® derived Cry9C inadvertently introduced into the test sample), or observed a reaction between other protein(s) present in the sample (not StarLink® Cry9C) with the antibodies of the ELISA plate test (cross-reaction).

F. Retesting Grain Samples Used to Estimate the Level of Cry9C in Exponent's Distribution 2

Some of the data on Cry9C residues generated by the ARS laboratory with ELISA plate testing and used by Exponent in its 2005 exposure analysis (i.e., to characterize distribution 2) might be interpreted as showing that low levels of Cry9C may have been missed on test and retest with the LFS assay, thus raising the question of whether as much as 11.4% of the corn grain supply could contain anywhere from 0.10 ppb to 2.73 ppb[28] Cry9C. Because of the variability observed at the LOD for the ELISA plate test, EPA asked the GIPSA laboratory to retest these samples in order to better understand the limitations of the ELISA plate test analytical method and the frequency with which Cry9C occurs in the grain supply.

Using the ELISA plate assay, the GIPSA laboratory retested 158 survey samples[26] originally tested by the ARS laboratory in Raleigh, NC and used to characterize distribution 2. All the samples were assessed by the GIPSA laboratory to be negative for the presence of the Cry9C protein, since none of the values obtained were above the LOD for the ELISA plate method. As noted above, the LOD for the ELISA plate test in the GIPSA laboratory was lower[29], and therefore able to detect for lower levels of Cry9C, than the LOD for this same test in the ARS laboratory.

G. Tumbled Sample Experiment: Can Cry9C be detected in non-StarLink® corn after it has been tumbled overnight with StarLink® corn?

To determine whether the handling (mixing, moving, pouring, etc, of the grain supply system, e.g., in the grain elevator) of non-StarLink® corn admixed with small amounts of StarLink® corn could produce dust or fragments that could be responsible for data indicating the presence of Cry9C (i.e., distribution 2), GIPSA conducted a "tumbled sample" experiment[26]. This experiment examined whether tumbling non-Cry9C kernels

[28] While these estimates are useful for Exponent's exposure assessment, they probably do not reflect the actual occurrence of Cry9C in the current grain supply.
[29] The GIPSA LOD was estimated to be 0.37 ppb from GIPSA June 20, 2007 Analysis and July 7, 2007 Addendum. "Analysis of individual corn kernels using ELISA-based technology to detect Cry9C in StarLink". The ARS LOD is estimated to be ~ 0.4 ppb from Whitaker/Slate November 14, 2006 Memorandum. "ELISA plate test results for Cry9C in corn grown prior to 1990."

spiked with either 1% or 10 % StarLink® kernels could generate a Cry9C positive test with the ELISA plate test once the individual StarLink® kernels were removed. GIPSA was able to show that while the 10% mixture did generate positive ELISA plate test results, the 1% mixture did not[26]. Given that under current commercial conditions where positive tests for StarLink® represent less than 0.01% of all tests and those that do test positive indicate very low levels, admixtures of StarLink® and non-StarLink® kernels are highly unlikely to occur at levels as high as 1% StarLink®. These results demonstrate that residual intact StarLink® kernels are unlikely to be the source of dust or fragments that might result in detectable Cry9C below 20 ppb on the ELISA plate test. This increases the probability that one of the other reasons discussed in Unit V.H. of this White Paper may explain the variability of results observed with the ELISA plate test at or near the LOD.

H. Interpretation of Test Results at or near LOD

Variability in test results at or near the LOD for Cry9C for both the LFS test and ELISA plate test appears to be a consistent feature of the testing that formed the basis of the Exponent 2005 study[15] and the tests on single corn kernels[25,26] grown prior to the development of StarLink®. This variability is expressed in both qualitative (positive or negative for the presence of Cry9C) and quantitative (how much Cry9C is present) terms. Qualitative differences can be observed for example:

- In the Exponent 2005 study, of the 1132 USDA FGIS samples that tested negative for Cry9C using the LFS test, 129 (11.4%) retested positive in the USDA ARS laboratory using the more sensitive ELISA plate test, with a mean of 0.26 ppb. (See Figure 3 of this White Paper). Of these 129 samples, 73 samples were positive in the two aliquots tested for each sample, while 56 of the 129 samples tested positive in one aliquot and negative in the second aliquot[30]. One hundred fifty-eight of the 1132 samples were retested by the USDA GIPSA laboratory[31] using the ELISA plate test. All of the retested samples gave negative results[32].

- Of the QCP samples used in the 2005 Exponent report (see Figure 2 of this White Paper) that tested positive in the field using the LFS test (341)[33], retesting a portion of them (56) resulted in 75% (42 of the 56) producing a negative result for the LFS test. When a subset (23) of the 42 that produced a negative LFS test result were assayed using the more sensitive

[30] Whitaker March 21, 2007 Memorandum. "ELISA plate results of Cry9C protein in commercial corn."
[31] GIPSA analyzed 158 survey samples originally assayed by USDA ARS. The retested samples contained all of the samples that had tested positive in distribution 2 in the ARS laboratory and some of the samples that tested negative in the ARS laboratory.
[32] These survey samples were analyzed blind by the GIPSA laboratory without any prior knowledge of the USDA ARS results.
[33] Table 1 of the Exponent November 16, 2005 Report. "Assessing exposure to Cry9C protein in StarLink® corn"

ELISA plate test, 6 produced a negative result and 17 a positive result with a mean of 7.6 ppb[34].

- In the ELISA plate test analysis of single corn seeds grown prior to the development of StarLink®[25], the USDA ARS laboratory obtained 16 positive results for Cry9C. Fifteen of these samples tested positive in one aliquot taken from a single sample but negative in the other, while one tested positive in both aliquots[35]. The average reading for all 16 samples was 1.36 ppb. Ten of the single seed samples were retested by the USDA GIPSA laboratory using the ELISA plate test and all retested negative for Cry9C[26].

Examining the quantitative results in these studies also reveals variability. Quantitative differences can be observed, for example, between the two aliquots that are routinely taken of a single sample for the ELISA plate test:

- In the ELISA plate testing of the USDA FGIS samples for the Exponent 2005 study[30], the two aliquots for each sample testing positive could vary by as much as 2.73 ppb (average difference 1.51 ppb, LOD ~ 0.125 ppb).

- In the ARS laboratory ELISA plate testing of the samples for the single seed analysis[25], the two aliquots for each sample could vary by as much as 9.78 ppb, with an average difference of 2.29 ppb (LOD ~ 0.4 ppb).

These observations indicate a degree of both qualitative and quantitative variability that would dictate caution in interpreting readings near the LOD for Cry9C of the LFS test and the LOD of the ELISA plate test [36].

In addition, the Agency also considered whether reproducible positives at very low levels between the LOD for the ELISA plate test and the LOD for the LFS test are actually detecting Cry9C from StarLink®, or whether the elevated optical density readings can be attributed to other sources, i.e., Cry9C from naturally occurring *Bacillus thuringiensis*, a cross-reacting protein (e.g., Cry1F), or some other source of elevated optical density readings (e.g., laboratory contamination or faulty reagents). To address these issues, the Agency considered the following two questions:

1) What is the likelihood that positive readings in the range between the LOD of the LFS test (~ 20 ppb) and the ELISA plate test (~ 0.115 ppb) could be due to StarLink® derived Cry9C?

[34] Table 2 of the Exponent November 16, 2005 Report. “Assessing exposure to Cry9C protein in StarLink® corn.”
[35] See Unit V.E. of this White Paper and the Whitaker/Slate November 14, 2006 Memorandum. “ELISA plate test results for Cry9C in corn grown prior to 1990.”
[36] Although the data generated at or near the LOD should be interpreted with caution, it is reasonable to use such information to estimate the upper bounds of human dietary exposure to Cry9C.

2) What are other potential sources of positive readings in the range between the LOD of the LFS test (~ 20 ppb) and the LOD of the ELISA plate test (~ 0.115 ppb)?

1) What is the likelihood that positive readings in the range between the LOD of the LFS test (~ 20 ppb) and the ELISA plate test (~ 0.115 ppb) could be due to StarLink® derived Cry9C?

There are three possible sources of StarLink® derived Cry9C in current grain stocks that could result in readings in the range between the LOD for the LFS test and the LOD of the ELISA plate test. The first is from actual StarLink® kernels from corn planted before 2001 still lingering in grain storage or transport systems. The second source would be from grain dust or other types of contamination containing fragments of StarLink® corn. The third possibility would be if the StarLink® *cry9c* gene introgressed into other corn lines. Available information casts doubt on each of these possible explanations.

If kernels of StarLink® corn still lingered in the grain transport or storage systems, and a single kernel was present in an 800 kernel sample tested by the ELISA plate test, the level detected should be approximately 20 ppb, given Cry9C residues in a StarLink® kernel average approximately 13,000 ppb. The levels detected in the ARS and GIPSA laboratories are well below 20 ppb, suggesting that the positive readings are not due to a whole kernel of StarLink® corn.

The results of GIPSA's 2007 "tumbled sample" experiment described in section V. G. of this White Paper addressed the question of whether dust or other residues could contribute to data indicating detection of Cry9C at a mean of 0.26 ppb in 11.4% of samples that first tested negative in a LFS test, as reported in the 2005 Exponent report[15]. GIPSA conducted its "tumbled sample" experiment to simulate conditions found in the corn grain handling system by tumbling non-StarLink® kernels spiked with either 1% or 10% StarLink® kernels overnight, removing all the StarLink® kernels and then testing with the ELISA plate test for the presence of Cry9C. GIPSA was able to show that while the 10% mixture did generate positive ELISA plate test results, the 1% mixture did not. This outcome indicates that in order for dust or residue from StarLink® kernels that are residual in the grain system to be the source of the positive Cry9C readings below ~ 20 ppb, the amount of StarLink® in the grain supply would have to be above 1%. The 2005 and 2006 Exponent reports and the GIPSA follow-up testing suggest it is highly unlikely on both a qualitative and quantitative basis that StarLink® comprises 1% of the grain supply. No StarLink® corn has been harvested since the 2000 growing season and extensive efforts have been used since then to remove StarLink® from the human corn grain supply. Even if such dust were originally present, as the years pass, it becomes increasingly unlikely that such possible sources of contamination can occur in quantities sufficient to be detected by the ELISA plate test. Rather, such residues would decrease over time as they are carried out of the system by non-StarLink® corn moving through the grain storage and transport system.

With regard to the possibility of the *cry9c* gene introgressing into other corn lines, any Quality Assurance/Quality Control procedures conducted by seed companies should further diminish the likelihood of *cry9c* gene introgression being a significant source of Cry9C in corn grain. Normally, seed companies conduct their own internal seed testing to meet labeling and other regulatory requirements and market needs. In addition to standardized testing protocols involving germination and varietal purity, tests may also be conducted for the presence or absence of a particular trait or genotype. These tests may include protein detection (e.g., LFS, ELISA plate) or DNA (e.g., polymerase chain reaction or PCR) tests.

2) What are other potential sources of positive readings in the range between the LOD of the LFS test (~ 20 ppb) and the LOD of the ELISA plate test (~ 0.115 ppb)?

In addition to considering whether the positive readings in the ELISA plate test below ~ 20 ppb in corn grain can be attributed to StarLink® kernels, fragments, or dust, EPA also evaluated other possible explanations. Specifically, EPA looked at whether the results could be attributed to:

- Contamination or variability of test reagents,
- Presence of other proteins that can cross-react with Cry9C antibodies in the ELISA test, and
- Naturally occurring *Bacillus thuringiensis* expressing the *cry9c* gene.

Potential contamination or variability of test reagents. Interpreting the results of any test, including the ELISA plate test for field corn samples tested for Cry9C derived from StarLink®, at or near the estimated LOD can be challenging and the interpretations involve significant uncertainty. Some of the challenge and uncertainty may be attributed to the fact that small variations in reagent production and protocol execution can substantially affect the results observed with very sensitive assay systems at their LOD. It is extremely difficult, if not impossible, to remove such variability from any assay system. Within the range where analytical variability is observed for a particular testing system, e.g., at or near the LOD of the LFS test and the LOD of the ELISA plate test for Cry9C, a positive result does not definitively demonstrate that the substance that the assay is testing for is present.

Presence of other proteins that can cross-react with Cry9C antibodies in the ELISA test. EnviroLogix' literature[37] for the QuantiPlate™ Kit for Cry9C test kit indicates there can be some cross-reactivity with at least four other Cry proteins (Cry1Ab, Cry1C, Cry2A, Cry1F). While three of these are detectable only at tens of thousands of ppb, Cry1F is detectable at levels of 66 ppb and legally present in corn commercially available today[38]. EPA has established a tolerance exemption for Cry 1F, and therefore

[37] Envirologix™ Catalog Number AP 008 QT "QuantiPlate™ Kit for Cry9C".
[38] In 2004, 2% of U.S. corn acres were planted to corn containing Cry1F. (From Biotechnology –Derived Crops Planted in 2004 – Impacts on US Agriculture. December 2005 S. Sankula, G. Marmon, E.

the presence of Cry1F in corn would not cause the corn to be adulterated[39]. It is unclear from the detection kit product insert if any other proteins potentially present in corn may cross-react and potentially interfere with the test for Cry9C. Further, from analytical method validation work at the EPA Environmental Science Center at Fort Meade, MD, there are suggestions that it may be difficult to obtain a corn sample completely free of Cry proteins. This would suggest that cross-reactive proteins could be common in the grain supply. However, without additional testing to establish the identity of the proteins interacting with the ELISA plate test antibodies targeted at Cry9C, it would not be possible to determine whether the protein interacting with the Cry9C-targeted antibodies was indeed Cry9C.

Naturally occurring Bacillus thuringiensis expressing the cry9c gene. Cry9C is a protein that is produced by a naturally occurring bacterium, *Bacillus thuringiensis*. It has been demonstrated that naturally occurring *B. thuringiensis* variants (i.e., not from commercially available insecticidal preparations) can be isolated from the surfaces of living plants[40,41]. Indeed, some scientists have argued that the observed population densities of *B. thuringiensis* on plant surfaces suggest that individuals of this species should be considered part of the natural microbial flora on the plant surface[40]. In addition, one of the richest sources of *B. thuringiensis* variants is grain storage and processing mills, and a higher percentage of *B. thuringiensis* isolates from North American maize dust are toxic to lepidopterans than to other insects[42,43,44]. The original natural source of the isolate that provided the *cry9c* gene was grain dust in the Philippines[45]. Thus, it is possible that pre-StarLink® seeds may have tested positive at these very low levels for Cry9C because of the natural presence on the kernels of *B. thuringiensis* producing Cry9C or a protein(s) cross-reactive with the Cry9C antibodies used in the ELISA plate test. Naturally occurring Cry9C from sources such as *Bacillus*

Blumenthal, National Center for Food and Agricultural Policy) at:
http://www.ncfap.org/whatwedo/pdf/**2004**biotechimpacts.pdf

[39] Additional information on Cry1F can be found at:
http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm
http://www.epa.gov/fedrgstr/EPA-PEST/2001/June/Day-06/p13837.htm

[40] Smith, R.A. and G.A. Couche. 1991. The phylloplane as a source of *Bacillus thuringiensis* variants. **Applied and Environmental Microbiology** 57:311-315.

[41] Frederiksen, K., H. Rosenquist, K. Jorgensen, and A Wilkes. 2006. Occurrence of natural Bacillus thuringiensis contaminants and residues of Bacillus thurginiensis-based insecticides on fresh fruits and vegetables. **Applied and Environmental Microbiology** 72:3435-3440.

[42] Bernhard, K., P. Jarrett, M. Meadows, J. Butt, D.J. Ellis, G.M. Roberts, S. Pauli, P. Rodgers, and H.D. Burges. 1997. Natural isolates of *Bacillus thuringiensis*: worldwide distribution, characterization, and activity against insect pests. **Journal of Invertebrate Pathology** 70:59-68

[43] DeLucca, A.J., M.S. Palmgren, and A. Ciegier. 1982. *Bacillus thuringiensis* in grain elevator dusts. **Canadian Journal of Microbiology** 28:452-456.

[44] Meadows, M.P., D.B Ellis, J. Butt, P. Jarrett, and H.D. Burges. 1992. Distribution, Frequency, and Diversity of *Bacillus thuringiensis* in an Animal Feed Mill. **Applied and Environmental Microbiology** 58:1344-1350.

[45] Lambert, B., L. Buysse, C. Decock, S. Jansens, C. Piens, B. Saey, J. Seurinck, K. Van Audenhove, J. Van Rie, A. Van Vliet, and M. Peferoen. 1996. A *Bacillus thuringiensis* insectidical crystal protein with a high activity against members of the family Noctuidae. **Applied and Environmental Microbiology** 62:80-86.

thuringiensis bacteria would not be subject to FIFRA and the tolerance requirements of FFDCA section 408. Therefore, it is important in assessing the reliability of the testing method to consider whether it could be detecting lawful, naturally occurring substances, instead of an unlawful pesticide chemical residue.

In sum, it would appear that caution should be exercised when interpreting ELISA plate test data when optical density readings suggest very low levels of Cry9C. Consideration should be given to the LOD of the testing method and known analytical variability for each testing method at or near the LOD. Consideration should also be given to whether there may be other proteins in the sample that may cross-react with the antibodies in the ELISA test for Cry9C to give a positive reading. Given the above analyses, when positive results are obtained for Cry9C at or near the LOD for a particular test method, confirmatory retesting would be prudent.

VI. Conclusions

The foregoing review leads EPA to conclude that the potential for exposure of the U.S. population, and significant subgroups in the population, to Cry9C in the U.S. food supply is currently so low that continued testing by grain handlers and millers of yellow corn grain for the presence of Cry9C provides no additional human health protection. This conclusion rests on careful consideration of the foregoing history of the scientific and regulatory review of StarLink® and the information accumulated since the last SAP meeting in 2001, particularly the new data and analyses concerning the levels of Cry9C currently in the food supply. Although uncertainties remain, four considerations indicate that risks are (and will remain) low and therefore the discontinuation of testing for StarLink® by grain handlers and millers is justified.

A. Dramatically Lower Exposure Levels

The company responsible for StarLink® (Aventis, later it successor entity SLLI) and EPA (as reviewed by the SAP) have all offered, at various time points, estimates of the amount of Cry9C from StarLink® to which the U.S. population may be exposed as a consequence of consuming food derived from corn grain. A comparison of these estimates – the estimates generated by EPA in 2000 and the estimates generated by Exponent (on behalf of SLLI) in 2005 and 2006 – provides very useful insights into the changes in the level of Cry9C remaining in human food as a result of coordinated efforts to contain and remove StarLink® from the human food supply.

A comparison of these estimates indicates that there has been a very large decline in exposure to Cry9C since 2000. Some of the differences between EPA's 2000 estimate and Exponent's 2005 and 2006 estimates may be attributable to differences in the ways in which the estimates were derived, but, by far, the most significant part of the difference results from the success of government and industry containment efforts in removing StarLink® from the human food supply. For example, in the most highly exposed group, the 99.9th percentile of exposure estimated by Exponent in 2006 (0.00747 µg/person/day) is about 8,000 times lower than the 99.9th percentile of exposure estimated by EPA in

2000 (57 µg/person/day). Even when Exponent in its 2005 study modified its assumptions in ways that would overestimate the levels of Cry9C in the corn grain supply (see Tables 5 through 8), the estimates showed levels that are thousands of time lower than the exposure estimated by EPA in 2000. In addition, it should be noted that Exponent's 2005 analysis, and by extension its 2006 estimate, are based upon residue values that fall for the most part at or near the LOD for ELISA plate testing. As discussed in Unit V.H. of this White Paper, the variability observed with these sensitive assay systems at or near the LOD and the fact that the positive ARS samples in distribution 2 when reassayed by the GIPSA laboratory tested negative, suggest that Exponent's 2005 and 2006 analyses could have resulted in estimates of far more Cry9C than was actually present in the corn grain supply in 2005 and 2006.

B. Relation of Exposure to Allergenic Risk

In understanding the significance of the foregoing comparisons, it is useful to examine how exposure may affect allergenic risk, since exposure to the allergen is an important consideration for both the sensitization and elicitation phases of the food allergic response. The first stage in the development of an allergic response to some component of food is a "sensitization" period where the immune system is primed to recognize a substance in food. The SAP said in 2000, shortly after StarLink® was first detected in food products intended for human consumption, that the amount of StarLink® in the human food supply has "a low probability to sensitize some individuals to Cry9C protein." This conclusion rested on the Panel's judgment that overall daily dietary exposure was relatively limited, perhaps, in all likelihood, below EPA's estimated upper bound of 57 µg/person/day. The Panel further commented that "[l]owering the levels of Cry9C in the food supply makes sensitization less probable." In July 2001, the SAP reaffirmed its November 2000 conclusion that there was a "low probability to sensitize individuals to Cry9C protein."

EPA believes that the exposures to Cry9C occurring in 2005 were (and continue to be) dramatically lower than were occurring in 2000 or 2001. The Agency agrees with Exponent's estimate developed for SLLI that mean exposure to Cry9C in 2005 is likely to be in the range of 0.00007 µg/person/day and that the 99.9th percentile of the exposure distribution is approximately 0.008 µg/person/day. These values are about four to six orders of magnitude below the corresponding levels of daily exposure estimated five years earlier in 2000 when the SAP concluded that the levels of Cry9C estimated to be in the food supply were so low that that there "was a low probability of allergenicity in the exposed population."

Nonetheless, the dose or cumulative exposure resulting in sensitization to any dietary protein is not generally known. At the same time, evidence of sensitization to an allergen alone does not necessarily correlate with clinical reactivity or responsiveness to an allergen, and doses causing sensitization may therefore not be relevant to assessing hazard from allergenic exposure. In this regard, the elicitation or reactive phase of the allergic response best represents the hazard from allergenic exposure, since associated doses result in clinical symptomatology and risk to human health. The recent experience with human consumption of corn since 2000 and the most recent exposure estimates for Cry9C in the corn supply provide solid evidence to evaluate the risk for allergenic reactivity posed by the current maximum exposures to Cry9C.

Although the dose of allergen necessary to elicit allergic responses (i.e., reactivity thresholds), has not been established for any known allergen, data describing minimal doses to elicit allergic responses, or lowest observed adverse event levels (LOAELs), to major food allergens (i.e. peanut, milk, egg, etc.)[46] in sensitive individuals are available. These data have shown the LOAELs for any allergen to induce at least mild, subjective allergic reactions to be in the 10 to 100 µg/meal range. Although these doses correspond to foods with multiple allergenic proteins rather than a single protein allergen and safety considerations based on these doses are subject to uncertainties due to challenge study design and materials as well as inclusion of sensitive populations, the reported LOAELS of major food allergens are at least 3 to 4 orders of magnitude higher than the amounts of Cry9C Exponent estimated in 2005 the most highly exposed individuals would consume (99.9th percentile of the U.S. population at 0.00821 µg/person/day).

Despite the fact that the levels of Cry9C estimated to be currently in the food supply are far below the lowest levels ever shown to provoke an allergic response in people allergic to known food allergens such as peanuts, milk and tree nuts[46], EPA cannot provide a definitive threshold for exposure to Cry9C below which it could be proven to pose no allergenic risk human health, if it were indeed a food allergen. Generally speaking, the only way to prove definitively that a substance is a human allergen is to identify an individual who exhibits an allergic response following exposure to the substance, and who has antibodies in his or her blood against the putative allergen. Such individuals are initially identified through incident reports in which a person claims to have experienced an allergic reaction in temporal association with exposure to the allergen. Given the limitations on incident reporting and the general absence of systematic follow-up, incident reporting is most likely to identify substances that are potent allergens affecting significant numbers of people. In 2000, the SAP observed that "[i]f corn-derived food products contain a highly potent allergen then allergic reactions should appear within a few years." (Emphasis added.) In 2001, FDA and CDC invested considerable effort to identify individuals who alleged they had allergic reactions associated with exposure to Cry9C, and investigate these claims. The information collected by FDA and CDC failed to identify any instance in which it appeared a person had experienced an allergic reaction to Cry9C. Since 2001, no other incidents reliably connecting allergic reactions and exposure to Cry9C have been reported to, much less documented by FDA or EPA. Moreover, although the exact prevalence of allergy to corn is not known, there is no evidence that allergies or reported allergic events to corn in general have increased in the years since StarLink® corn was introduced into the food supply.

Given that exposure has decreased by an estimated 4 to 6 orders of magnitude since 2000, it follows that current levels are unlikely to lead to sensitization of even small

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[46] Report "Approaches to establish thresholds for major food allergens and for gluten in food" at http://www.cfsan.fda.gov/~dms/alrgn2.html

numbers of individuals. In EPA's view, the reduction of exposure that has occurred since 2001 means that it is unlikely anyone will become allergic (i.e., sensitized) to Cry9C as a result of the low level of exposure encountered in today's food supply. Moreover, should there be any individuals who were sensitized to StarLink® derived Cry9C during 2000 and 2001, these individuals are now unlikely to be exposed to any more than very low levels of Cry9C, if any at all. There is little or no reason to expect the very low levels of Cry9C residues estimated to be in the food supply at this time to cause serious adverse allergic reactions.

In sum, estimated levels of Cry9C exposure are orders of magnitude lower than the minimal eliciting doses reported for most major food allergens. Consistent with the SAP's reasoning, the absence of reports associating Cry9C with allergic reactions, while certainly not conclusive, provides supporting evidence that Cry9C at the levels present in the food supply from 2001 to the present is not triggering significant, if any, allergic responses. We therefore conclude that there is little or no reason to expect the very low levels of Cry9C residues estimated to be in the food supply at this time to cause serious adverse allergic reactions.

C. Current Exposures Lower than 2001 Wet-Milling Estimates

EPA's estimate of potential exposure to Cry9C through consumption of food products containing ingredients derived from wet-milled corn grain and EPA's comments about the levels of allergenic risk posed by such exposure create a context that is useful in evaluating the potential allergenic risk posed by the levels of Cry9C being detected in the human food supply since 2005. Because EPA judged the levels of Cry9C coming from consumption of food ingredients from wet-milling in 2001 to pose no risk to public health, it follows that as the potential for exposure to Cry9C from all sources as estimated in 2005 and 2006 is comparable or lower, such exposure also poses no risk to public health. The wet-milling process removes virtually all protein content from the various processed fractions produced for human food consumption, and as analyzed in the 2001 White Paper, since in StarLink® grain only a very small percentage of total protein is Cry9C, the total daily exposure that could result from consumption of the various wet milling fractions would be very low. EPA's upper bound estimate for exposure to Cry9C from wet-milled food ingredients in 2001 for the 99.5th percentile consumer was 0.019 µg/person/day. The Agency's 2001 White Paper on the effects of wet-milling concluded that "it is reasonable to conclude that there is virtually no Cry9C protein in wet milled products and that there is no likely health concern for the public associated with the consumption of any food fraction produced by wet milling of corn as long as reasonable steps are taken to ensure that StarLink® is not diverted into wet milling." In July 2001, EPA reaffirmed this conclusion saying, "there is no public health risk from eating products manufactured from StarLink corn through the wet-milling process, provided that corn utilized in the wet-milling process does not contain significant levels of StarLink."

In Exponent's 2005 report, the levels of exposure to Cry9C from all sources in 2005 are estimated to be lower than the exposure to Cry9C from wet-milled products alone in 2001. Specifically, a comparison of the levels occurring at the 99.5th percentiles of the two relevant estimates shows about a six fold difference: 2005 Exponent estimate of 0.003 µg/person/day vs. 2001 EPA estimate of 0.019 µg/person/day. Even the highest values estimated in any of the sensitivity analyses performed by Exponent in 2005 were lower than the levels estimated in 2001 in food derived from wet milling. (See Tables 5 through 8). It is important to recognize that EPA and Exponent used different methodologies to develop estimates of Cry9C exposure. Thus, any comparison will not produce a highly precise estimate of the relative levels of exposure at these different time points. Nonetheless, EPA believes that the EPA 2001 and Exponent 2005 analyses are sufficiently comparable in methodology to conclude that 2005 estimate of exposure to Cry9C from all sources lies in the same range as (or falls below) the levels of Cry9C estimated exposure from wet-milled products alone in 2001. Further, while some portion of any apparent difference in estimated exposures may result from different estimation methodologies, EPA believes that, by far, the most significant factor accounting for the different estimates is the virtually complete removal of StarLink® grain from the food supply.

In 2006, Exponent provided additional information updating its estimates of exposure to Cry9C. The 2006 information indicates that detections of Cry9C from October 2005 through September 2006 occurred less frequently than they did in 2005. Using this 2006 information, Exponent developed estimates of potential exposure to Cry9C in the diet. Not surprisingly, these estimates indicate that exposures to Cry9C in 2006 (0.00288 µg/person/day for the 99.5th percentile of the U.S. population (See Table 10)) are lower than in 2005. As the 2001 White Paper judged the upper bound of exposure for wet milled fractions not to pose a risk to public health or to raise a likely health concern, the levels of Cry9C estimated to be in the corn grain supply today should similarly be regarded as posing no risk to public health.

D. Terminating Diversion of Grain Testing Positive for Cry9C from the Human Food Supply Negligibly Affects Exposure

At EPA's suggestion, in 2006 SLLI directed Exponent to perform some additional analysis to provide a quantitative assessment of the impact on exposure to Cry9C of ongoing monitoring and diversion efforts. Exponent performed a probabilistic exposure assessment estimating the effect on human dietary exposure of the efforts to identify and divert lots of corn testing positive on the LFS test to domestic animal feed or industrial uses.

When instituted in 2000, the monitoring program recommended by FDA resulted in the identification and diversion of numerous shipments of corn testing positive for Cry9C to domestic animal feed or industrial uses. From 2000 onward, millions of bushels of grain have been redirected as a result of the StarLink® containment program. These actions reduced the levels of Cry9C in the human food supply and lowered the likelihood that, if StarLink® derived Cry9C were a human allergen, any individual would receive enough exposure to become sensitized, and that, if any person did develop an allergy to StarLink® derived Cry9C, the sensitized individual would not be exposed to sufficient levels of the allergen to produce an allergic response.

The analysis that Exponent performed in 2006 comparing estimates made assuming that lots testing positive for Cry9C were diverted from the human food supply to acceptable uses to estimates made assuming that lots testing positive would remain in the food supply (Table 11), shows that the continuation of monitoring all shipments of corn grain coming into dry milling facilities and masa operations as recommended by FDA is no longer significantly reducing exposure to Cry9C. The comparison shows that, because positive tests are so extraordinarily rare, monitoring and diversion of grain from the human food supply has no measurable effect (about 0.000017 µg/day difference) on the levels of exposure received by the overwhelming majority (99.5th percentile) of consumers. The greatest difference in estimated exposure was an additional 0.000185 µg/day, and that difference affected only 0.1% of all consumers (i.e., 99.9th percentile). Therefore, the Agency concludes that continuation of the monitoring program likely is having no effect on the potential for exposure and thus having no effect on the potential for allergenic risk.

VII. Summary Recommendation

In light of the above analyses EPA recommends:

- FDA should withdraw its guidance for dry milling facilities and masa operations that recommends sampling and testing yellow corn and dry-milled yellow corn shipments intended for human food use for Cry9C protein residues.