WHITE PAPER
ON THE POSSIBLE PRESENCE OF CRY9C PROTEIN IN PROCESSED HUMAN FOODS MADE FROM FOOD FRACTIONS PRODUCED THROUGH THE WET MILLING OF CORN

I. Introduction

StarLink is a variety of *Bt* corn that has been genetically engineered to produce a protein, Cry9C, intended to be toxic to certain insect pests of corn. Following a thorough scientific review of the safety of this product, EPA concluded that, other than an unresolved issue regarding the potential for Cry9C to pose an allergenic risk to humans, StarLink would pose no risks to public health or the environment. Therefore, EPA issued a registration for the Cry9C protein and the genetic material necessary for its production (called a plant-pesticide) in 1998 to AgrEvo (now Aventis CropScience). EPA limited the registration by requiring that all StarLink corn only be used 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 the Cry9C protein.

Because of Aventis' continuing interest in obtaining approval for use of StarLink 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 Scientific Advisory Panel (SAP), on February 29, 2000 regarding Cry9C protein. (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.) The February 29, 2000 SAP report stated that with the data available, it could not be determined whether or not Cry9C is an allergenic protein.

In September 2000, *cry9c* DNA was detected in a finished food product - taco shells. Subsequently, the DNA and protein have been found in corn grain and other corn products in the food supply. These detections indicated that, despite the EPA restrictions, some quantities of StarLink corn had directly entered the human food chain.

On October 12, 2000, Aventis requested that the registration for their StarLink corn product be voluntarily cancelled. As a result, StarLink corn is not authorized for planting in future years. On October 25, 2000, Aventis amended its petition for a food tolerance exemption under the Federal Food, Drug, and Cosmetic Act (FFDCA) to ask for a temporary tolerance of four years to cover any Cry9C protein and *cry9c* DNA that may be present in human food made from StarLink corn planted in 1998, 1999, and 2000. Aventis submitted additional information with its petition to support its contention that the Cry9C protein posed no allergenic risk to public health. EPA convened another SAP meeting on November 28, 2000 to consider the question of the potential of the Cry9C protein to be an allergen, whether there is an adequate amount of the protein in corn to cause sensitization, and what amount of Cry9C might be in the human food supply if this time limited tolerance exemption were to be approved. More information including the Aventis submission, EPA's papers for SAP review, background information, and the SAP final reports can be found on the following web sites:

http://www.epa.gov/pesticides/biopesticides/cry9c/index.htm
http://www.epa.gov/scipoly/sap/index.htm

The final report from the November 28, 2000 SAP meeting was issued on December 5, 2000 which expressed the consensus of the Panel that while Cry9C has a "medium likelihood" to be an allergen, the combination of the expression level of the protein and the amount of corn found to be commingled poses a "low probability" to sensitize individuals to Cry9C. The Panel report noted that the likelihood of the protein being detected in different corn products varied considerably, especially depending 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 called on EPA to only include in our dietary assessment those ingredients from corn that contain protein after processing. The SAP report states that items such as corn syrup, corn oil, and starch contain virtually no protein.

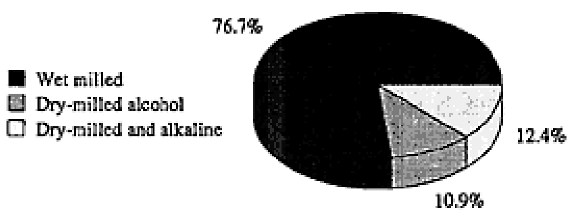
EPA did not include corn syrup and corn oil in its dietary assessment presented at the November 28, 2000 SAP meeting because protein is absent or virtually undetectable in these food products. However, the Agency has decided to further review wet milling methods to address potential dietary exposure to the Cry9C protein. EPA's review is based on published literature, comments from the SAP members during the November SAP meeting, software on dietary exposure, and information from corn industry representatives. EPA examined some of the raw data from an industry performed starch study, but did not examine any of the other raw data forming the basis of the information and conclusions from the cited literature and industry representatives. In addition, EPA has completed a review data recently submitted by Aventis which describes results of tests performed on food products made with 100% StarLink.

Field corn that is made into processed foods for human consumption undergoes milling. There are two primary types of milling: "dry milling" which produces flour and meal primarily and "wet milling" which produces high fructose corn syrup, oil, starch, some animal feed products, and ethanol primarily. Corn products produced by wet milling contain varying levels of protein. Those products intended for human food consumption contain no or extremely low levels of intact protein. The exception is the use of one protein, corn zein, specifically extracted from corn. Corn zein will be discussed further in Section III. In contrast, products intended for animal feed may contain high levels of protein. The wet milling process effectively separates these products and industry standards control protein content in food products to a very low or undetectable level. The more simple dry milling process does not separate protein from products intended for human consumption, although additional processing such as alkaline treatments and cooking may also affect the level of protein in the finished food. The focus of this paper is on the wet milling process, although dry milling and masa processing are briefly discussed to provide a more complete understanding of the milling processes.

II. Corn Milling

Nearly 2 billion bushels of corn are typically produced annually in the United States for food and other industrial purposes. This represents approximately 20% of the corn grown in the U.S. annually with the remaining 80% typically used for animal feed. The majority of corn earmarked for food or industrial use is subjected to wet milling (Figure 1). The remaining corn is subjected to either dry milling or alkaline cooking plus dry milling (Masa processing). In general, only yellow corn is used for wet milling, while both white and yellow corn are used in dry milled products. The predominant type of yellow corn used in wet milling is a "dent-type" corn. Approximately one-third of the yellow corn used for starch production in the wet milling process is a "waxy-type" maize. StarLink corn is a "dent-type" of maize, not the waxy-type.

Figure 1. Percentage of Corn Used in Each Milling Process



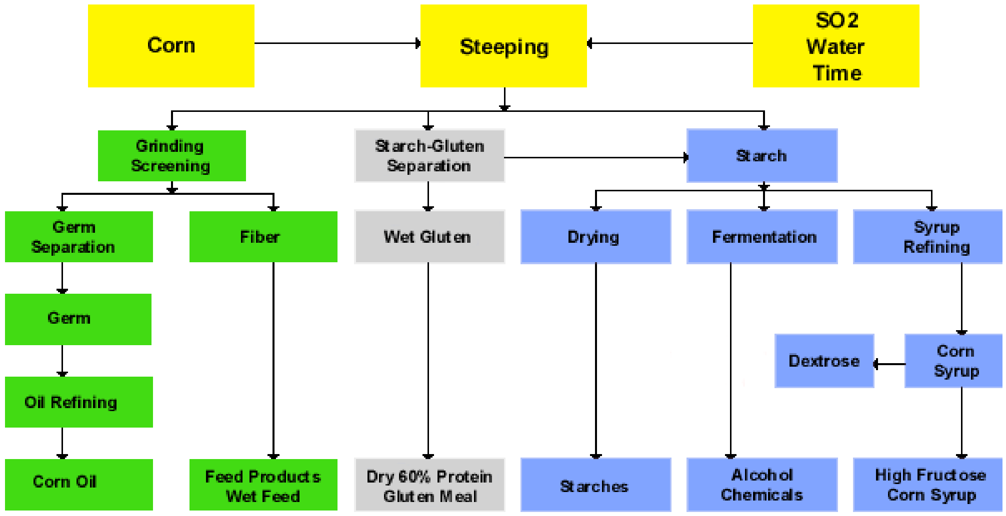
Source: http://www.ianr.unl.edu/pubs/fieldcrops/g1115.htm

A. Corn Wet Milling

The wet milling process involves a series of steps by which corn is separated into various components, which are then further processed and/or used for animal feed. The basic steps for wet milling include steeping, germ separation, fine grinding, starch separation, fermentation, and syrup conversion. Corn processed by wet milling is typically separated into 5 basic components: starch, germ, gluten, fiber and steep liquor (Blanchard, 1992). By-products of wet milling along with corn germ and most corn gluten are used for animal feed products. A very small amount of corn gluten is subjected to acid hydrolysis resulting in amino acids and/or short peptide units called hydrolyzed vegetable protein (HVP).

The wet milling process involves a series of steps which produce the various fractions described below (Corn Refiners Association, 2000; AAC, 1998; Blanchard, 1992; Jackson, 1996; May, 1987). Figure 2 provides a basic overview of the wet milling process. Corn received at a refinery is inspected and cleaned twice to remove cob, dust, chaff and foreign materials prior to steeping.

Figure 2. Corn Wet Milling Process



Source: Minnesota Corn Processors, LLC: http://www.mcp.net/info/wetmill.html

Wet Milling Process (as described: Corn Refiners Association, 2000; Minnesota Corn Processors, 1999):

1. Steeping:
Steeping takes place in stainless steel steep tanks which hold approximately 3,000 bushels of corn each. Corn is stored in these tanks for approximately 30 to 40 hours in 50° C soaking water which contains approximately 0.1% sulfur dioxide. During the incubation period, kernel moisture levels increase to between 15 and 45%, which also results in an increase in kernel size (up to 2X). As the corn is stored in the mildly acidic steep water (sulfur dioxide reacts with water to form sulfurous acid which is a strong reducing agent) the disulfide bonds in corn gluten loosen, which allows for separation of protein and starch. After steeping, the corn is coarsely ground to break the germ loose from the other components. The steep water is condensed to capture nutrients and this water is used for animal feed and future fermentation processes. The resulting ground corn is contained in a water slurry which flows to cyclone germ separators.

2. Germ Separation:
Corn germ contains approximately 85% of the oil found in corn. The cyclone separators, which are similar to centrifuges, spin the low density corn germ out of the slurry and pump the germ onto screens where the germ is washed repeatedly to remove any residual starch. The germ is finally subjected to a combination of mechanical and solvent processes which extract the oil from the germ. The resulting germ residue is saved to be used as a component of animal feed.

3. Fine Grinding:
The corn and water slurry are moved from the germ separator into an impact or attrition-impact mill to release the starch and gluten from the fiber in the kernel. The suspension of starch, gluten and fiber flows over fixed concave screens which catch fiber but allow the starch and gluten to pass through. The fiber is then collected, slurried and screened again to reclaim any residual starch or protein, then piped to the feed house to be used as a major component of animal feed. The starch-gluten suspension (mill starch) is piped to the starch separators.

4. Starch Separation:
Mill starch is passed through a centrifuge which allows for the gluten to be spun out, mostly for use in animal feed (gluten has a lower density than starch). At this point, the starch has only approximately one to two percent of protein remaining. The starch is diluted 8 to 14 times, rediluted and washed again in hydroclones to remove the last trace of protein and produce high quality starch (usually > 99.5% pure). Most of the corn starch is converted into corn syrups, dextrose, high fructose corn syrups, crystalline fructose and alcohol. Some of this starch is dried and marketed as unmodified corn starch and some is modified into speciality starches.

5. Syrup Conversion:
Starch is suspended in water and liquified in the presence of acid and/or enzymes. The resultant product is a low-dextrose solution. The solution is enzymatically-treated further to continue the conversion process of starch into syrup. Throughout the process, refiners can halt acid or enzyme actions at necessary points to produce the proper mixture of sugars (e.g., dextrose and maltose) for syrups. The syrup is refined in filters, centrifuges and ion-exchange columns and excess water is evaporated. Syrups are sold directly, crystallized into pure dextrose, or processed further to create high fructose corn syrup.

6. Fermentation:
Corn starch is also used to produce feedstock suitable for traditional yeast or bacterial fermentation methods. Enzymes are used to modify corn starch to produce the feedstock and the resulting fermentation product is ethanol. Alcohol production by wet milling accounts for approximately 306 million bushels of corn annually. This ethanol product is distilled to remove excess water and sold for use in industry and beverages. A by-product of the fermentation, carbon dioxide, is also sold to beverage manufacturers to be used in carbonated beverages.

B. Corn Dry Milling and Alkaline Processing

Dry milling for food use (including Masa processing) represents approximately 165 million bushels annually in the U.S. Both white and yellow corn are processed by dry milling to produce food products, with approximately 50 million bushels (about 30%) of this corn being white corn (David Shipman, Personal Communication, 2000). Dry milled corn is produced by a roller milling process. As in the wet milling process, the corn is initially cleaned. Once clean, the moisture content of corn is raised to about 20%. The corn germs are then removed for oil extraction and the remaining corn is ground and sieved into many fractions which vary in particle size and composition. The primary products of dry milling are flour, cornmeal and grits. Additional products include corn bran and feed mixtures. These products are used in brewing, foods, building products (binders), fermentations (pharmaceuticals and fuel), and animal feeds. Dry milling for alcohol production accounts for approximately 161 million bushels annually in the U.S.

Alkaline-cooked (Masa processing) corn is used in tortillas, tortilla chips, corn chips and other similar items. Whole kernel corn is cooked in near-boiling water containing 1% lime for approximately 20 minutes. The corn is allowed to soak for 8-12 hours (steeping). The corn is then drained from the steep water and washed with clean water to remove excess lime and the pericarp which has been loosened. The washed corn is now at about 45-50% moisture and is subjected to stone grinding to form a dough. If the dough is formed into strips and fried, corn chips are produced. If the dough is formed into thin pancake-like sheets and baked, corn tortillas are produced. If the baked tortillas are subsequently fried, tortilla chips are produced.

III. Protein Content of Fractions Resulting from Wet Milling

The wet milling process effectively separates protein-containing and non-protein-containing products. Table 1 below provides the approximate percentages of protein found in each of the various wet-milled fractions/products.

Table 1. Protein Contents and Uses of Products of Wet Milling

Fraction	Approximate Percent Protein Content[1]	Uses
Steep Liquor (condensed)	45-48% Protein	Animal Feed
Corn Germ	20%	Animal Feed
Bran/Gluten Feed	18-22% Protein	Animal Feed[2]
Gluten Meal	≥ 60% Protein	Animal Feed[2]
Starch	0.3-0.35% Protein (high amylose corn - up to 1%)[3]	unmodified corn starch, speciality corn starch, corn syrups, and dextrose
Syrup	Not Detectable[4] (made from corn starch)	pure dextrose, corn syrup, and high fructose corn syrup
Alcohol	Not Detectable[4] (made from corn starch)	ethanol
Corn Oil	Not Detectable	cooking or salad oil

[1] Source: Blanchard,1992; Kyd Brenner, 2000
[2] Most corn gluten is used for animal feed. Some is treated to extract corn zein and some is subjected to acid hydrolysis to produce hydrolyzed vegetable protein as described in Section III. B.
[3] Cry9C protein has not been engineered into high amylose and waxy corn varieties
[4]Further processing of corn starch removes remaining protein

Preliminary studies have been performed to determine if any Cry9C protein was present in starch samples (Charles Conner, Personal Communication, 2001). These studies were done using ELISA test kits made by both the EnviroLogix (EnviroLogix, Inc) and SDI (Strategic Diagnostics, Inc) to detect Cry9C protein. In addition, food products made from 100% StarLink corn have been tested by Aventis using an ELISA protocol developed by Aventis and the EnviroLogix ELISA test kit. This data has been formally reviewed by the Agency (MRID# 453866-03).   The FDA has recently validated the EnviroLogix test kit used by Aventis to confirm that it is adequate to detect Cry9C protein in processed foods. Thus far, as expected, the data indicate that proteins levels are extremely low to non-detectable in corn wet-milled fractions produced for food use.

In addition, a report on DNA analysis in maize (corn) starch and starch hydrolysates (various corn sugars coming from the wet milling process) shows that corn DNA and DNA from *Bacillus thuringiensis* (*Bt*) engineered into corn can be detected in starch, but not in the starch hydrolysates (AAC, 1998). The *Bt* DNA could be detected in wet milling products such as the germ and fibers where corn proteins also are commonly found. However, five independent laboratories were unable to detect any corn or *Bt* DNA in maltodextrin, glucose syrup produced by three separate processes, crystalline dextrose, and crystalline fructose. In addition, no DNA was detected in refined corn oil. Such tests usually detect fragments of DNA and the heating and other milling process are likely to degrade and denature any proteins. The authors were unable to obtain analytical methods for the detection of the *Bt* proteins and therefore could not conduct these analyses. In general, a DNA test is more sensitive than a test for protein. Although not itself a measure of protein content, the use of highly sensitive DNA detection technology and the inability to detect cry9c DNA in corn syrup and corn oil is supportive of the high purity of these products and the lack of cry9C protein in them. Corn proteins are found in food grade starch at very low levels. Whole kernel corn contains about 8.5 to 12 % protein, but food grade starch has protein levels two orders of magnitude lower at 0.3 to 0.35% (Kyd Brenner, Personal Communication, 2000; Corn Refiners Association, 2000b; Blanchard, 1992).

A. Animal Feed Products

Four major animal feed products are produced from different combinations of steep water, corn germ residues, fiber and corn gluten (Corn Refiners Association, 2000). These products include gluten meal, gluten feed, corn germ meal and condensed fermented corn extracts (steep water). Each of the products contains a relatively high percentage of protein. Corn gluten meal supplies vitamins, minerals and energy in such products as poultry feed. Steepwater is a liquid protein supplement for cattle and is also used as a binder in feed pellets. Corn gluten feed provides protein and fiber for beef cattle. All of these products are strictly limited to animal feed use and would not be present in the human food supply.

B. Gluten-Derived Products

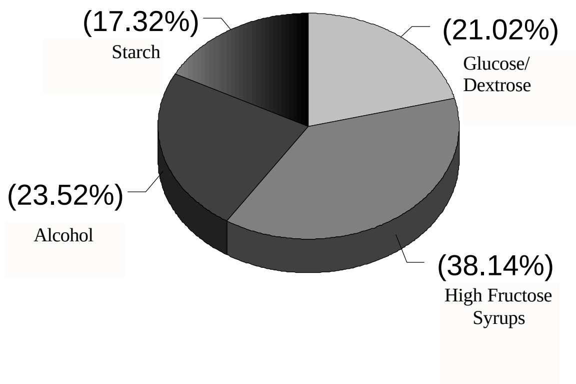
Corn zein is an insoluble protein which is contained in the corn gluten fraction (see http://www.arserrc.gov/es/zeinextratech.htm). Corn zein is used as a glazing and coating agent in the food and pharmaceuticals industries. In contrast, Cry9C is a water soluble protein. The high volumes of water used in the extraction/purification process to obtain zein should therefore eliminate the presence of any Cry9C and other water soluble proteins from corn zein.

A very small amount of corn gluten is subjected to acid hydrolysis resulting in a hydrolyzed vegetable protein (HVP). Acid hydrolysis using extremely concentrated (up to 6 normal) hydrochloric acid for 24 hours degrades a protein into its constituent amino acids and/or short peptides. These HVPs are more commonly produced from soybeans and wheat. Even though corn, wheat and soybeans are known as food allergens, there have been no well documented cases of adverse reactions to any hydrolyzed vegetable protein (Taylor, Personal Communication 2001).

C. Starch and Starch-Derived Products

Starch and starch-derived products account for approximately 74% of the products obtained by wet milling as a percentage of the raw corn (May, 1987). Figure 3 shows the approximate distribution of food use of starch-related products resulting from wet milling.

Figure 3. Food Use of Starch Resulting from Wet Milled Corn



Sour
ce:http://www.ianr.unl.edu/pubs/fieldcrops/g1115.htm

The amount of corn used for starch and starch-derived chemicals approaches nearly 1 billion bushels annually in the U.S. with approximately 250 million of these bushels used for corn starch production (Table 2). About 15% (37.5 million bushels) of this corn starch is for food and pharmaceutical use and the remaining 85% (212.5 million bushels) is designated for industrial use (David Shipman, Personal Communication, 2000). Approximately 33% of the food starch market is comprised of corn starch made from waxy maize (Cry9C has not been bred into this speciality corn [Torres, Personal Communication, 2000]). For corn starch produced from most corn, residual protein levels must be below 0.5% according to industry standards (NAS food chemicals CODEX [Brenner, Personal Communication, 2000]). Generally, this level is reduced even further to between 0.3-0.35% (Kyd Brenner, Personal Communication, 2000; Corn Refiners Association, 2000b; Blanchard, 1992). The only exception to these levels of protein in starch occurs when the starting material is high amylose corn. High amylose corn is a minor specialty corn (Cry9C has not been bred into this speciality corn), where protein levels must be below 1.0% in the finished starch. In addition, only about 0.01% of the protein in finished starch is water soluble protein due to the large number of water washes in the wet milling process (Corn Refiners Association, 2000a). Again, Cry9C is a water soluble protein and any Cry9C that remained in starch would be as a fraction of this 0.01% soluble protein. Because of the low levels of soluble protein found in corn starch, the levels of protein in corn starch-derived products would be less than the 0.01% and are undetectable in such products as corn syrup and alcohol because of further processing. In addition to food uses, starch is routinely used as an adhesive, for manufacture of papers, wallboard, adhesives, anticaking agents, dusting powder, thickening agents and as a filler for pharmaceuticals (Jackson, 1996).

Table 2. Summary of Corn Disposition Going Into Food Starch

Corn Use	Approximate Amounts
Total Corn Wet Milled Annually	1.35 Billion Bushels
Portion of Wet Milled Corn Bushels Used to Make Starch and Starch-Derived Products	1 Billion Bushels (74%)
Portion of Starch Output Used Specifically to Make Corn Starch	250 Million Bushels (25%)
Portion of Corn Starch Used for Food and Pharmaceuticals	37.5 Million Bushels (15%)

D. Corn Oil

As described earlier, corn germ contains approximately 85% of the oil found in corn. Once corn germ is separated as part of the wet milling process, corn oil is further refined (Rich Torres, Personal Communication, 2000). Crude oil is degummed in the presence of phosphoric acid which removes proteins, phospholipids, gums, etc. Following degumming, the oil is alkaline treated which removes fatty acids, neutralized and bleached. Finally, the oil is deodorized which removes residual proteins/amino acids and color bodies, resulting in a refined oil product. Approximately 50% of U.S.-produced corn oil is used for cooking or salad oil and another 25% is used for corn oil margarine (Corn Refiners Association, 2000). Through the corn oil refining process, proteins are removed and are not detectable in food grade corn oil (Brenner, Personal Communication, 2000; Torres, Personal Communication, 2000). In addition, *Bt* DNA could not be detected in the corn oil (AAC, 1998).

IV. Dietary Exposure to Corn Starch

As noted earlier, the FIFRA Scientific Advisory Panel met on November 28, 2000 to offer advice on EPA's "Assessment of Scientific Information Concerning StarLink Corn." The SAP's final report of that meeting contains several references to their expectation that human food fractions produced from wet milling of corn will not contribute significantly, if at all, to potential human exposure to Cry9C protein. For example, the SAP wrote: "As is entirely appropriate, both the Agency and Aventis count only those ingredients that contain protein after processing in assessing dietary exposure. Thus foods containing corn bran and corn endosperm are counted, while corn syrup, corn oil, starch and other food forms made from corn grain are not counted since they contain virtually no protein." (SAP Report p.21).

Because corn starch used in human food is likely to have a very low level of corn protein (typically about 0.3% total protein) and some of that total protein could be Cry9C, EPA has performed a quantitative assessment of the potential for human exposure to the Cry9C protein from corn starch. EPA has developed a new estimate of exposure from consumption of food containing corn starch. This estimate follows the methodology that EPA used to produce its earlier estimates of exposure presented to the (SAP) for its November 28, 2000 meeting and new information gathered since November. (The original paper (referred to as the November Exposure Assessment) may be found at:
http://www.epa.gov/scipoly/sap/2000/november/prelim_eval_sub102500.pdf.)

EPA's November Exposure Assessment depended on the three variables: (1) the amount of corn product consumed, (2) the percentage of the corn used in making a food item that was StarLink, and (3) the level of Cry9C in the StarLink corn portion of the food. The SAP agreed with EPA's basic approach and, therefore, EPA has used a similar approach in calculating an estimated exposure to Cry9C protein solely from corn starch. The variables were modified based upon the available information for corn starch produced by wet milling and starch consumption data from TAS-DIET software leased by FDA. EPA has changed its "mixing" assumptions, i.e., the assumptions regarding the percentage of commingled corn that is StarLink, by including the assumption that no more than 0.125% of the corn grain used in making corn starch is StarLink. This value reflects EPA's assumption that millers are using the testing methodology recommended by GIPSA to detect StarLink in incoming grain; this testing regimen has a limit of detection of 1 kernel in 800 or 0.125%. It has been reported that wet milling facilities are testing incoming corn following the GIPSA recommendations. EPA cannot confirm to what extent, past and present, that this method has been employed and therefore the percentage of starch that may contain Cry9C could vary depending upon the testing regimen. The EPA currently does not have any quantitative information on the number of mills employing StarLink screening methods, nor their protocols. Those facilities that may test at higher than the 1 in 2400 method will likely achieve a greater confidence level that StarLink is not present and those testing fewer corn kernals will likely achieve a lower confidence level.

EPA's exposure estimates were calculated as follows:
Exposure = [daily corn starch consumption] X [portion of the corn used to make corn starch that is StarLink] X [portion of corn starch that is corn protein] X [portion of corn protein in corn starch that is Cry9C]

Consumption of cornstarch was estimated using the TAS-DIET software (see Table 3), which is based on the USDA 1989-91 Continuing Survey of Food Intakes by Individuals (CSFII). Although consumption of cornstarch, per se, is not reported in the CSFII, TAS-DIET allows for estimating consumption of raw agricultural commodities (RACs) from the survey food codes. Cornstarch consumption was estimated by using the RAC code for corn grain endosperm only (which represents the ingredient cornstarch as well as cornmeal and corn flour) but restricting the survey food codes to only those that would likely contain cornstarch as opposed to cornmeal or corn flour.

In estimating the potential exposure to Cry9C protein, due to the consumption of corn starch, EPA examined the exposure of the general US population, as well as of various age groups of infants and children. (See Table 3.) Because data indicated that Hispanics generally consumed higher levels of many corn flour-based and corn meal-based foods than the general population, the November Exposure Assessment also estimated exposure to Hispanics and different age groups of Hispanic children. Data, however, do not show that the Hispanic population is more likely to consume corn starch produced through the wet milling than is any other portion of the US population. So while EPA's November assessment specifically considered these subpopulations, this assessment of exposure to corn starch produced from the wet milling process does not include specific assessments for Hispanic subpopulations.

Table 3: Per Capita Estimates of Daily Consumption of Corn Starch in g/day.

Population Subgroup	95^{th} Percentile	99^{th} Percentile	99.5 Percentile
US Population	20	57	81
All infants (<1 year)	7	11	12
All children, 1-6 years	6	15	20
All children, 7-12 years	9	25	33

Source: TAS-DIET software based on the Continuing Survey of Food Intake by Individuals from 1989 to 1991.

EPA's estimate of the portion of the corn used to make corn starch is based on information about the extent to which StarLink corn may have become commingled with non-StarLink corn. The November Exposure Assessment explains the basis for assuming a "mixing rate" of 1.2% and 1.5% for 1999 and 2000 crop years, respectively. In order to indicate an upper bound estimate and to afford a basis for comparison to the other exposure estimates in the November Assessment, EPA calculated exposure using the 1.5% and 1.2% "mixing rates." The resulting estimates appear in Tables 4 and 5, respectively. As discussed at length in the December SAP report, however, these values are likely to overestimate very significantly the potential exposure, because, among other reasons, milling facilities began to screen incoming corn for the possible presence of StarLink and to redirect corn testing positive.[1] Thus, EPA has added a third "mixing rate" value, 0.125%, to reflect the impact of following the GIPSA guidance for testing in wet milling facilities for the possible presence of StarLink. The GIPSA guidance directs that all corn grain coming into a wet milling operation be sampled and tested for the presence of StarLink corn. The guidance calls for taking 3 samples of 800 kernels and testing each sample separately. The analytical method is capable of detecting 1 StarLink kernel in 800, and therefore the testing regimen has a limit of detection of 1 in 800 kernels or 0.125%. The exposure estimates in Table 6 assume that the corn starch containe StarLink. Even these calculations are likely to be an overestimate because the incoming corn is likely to contain somewhat less StarLink than the LOD. See Brassard, 2001

Finally, in Table 6 EPA refined the estimate for the level of dietary exposure to Cry9C protein in corn starch. This was done using data on the amount of Cry9C in corn starch made from 100% StarLink corn, reported in Aventis' latest data submission (MRID 453866-03). This analysis shows that corn starch made from 100% StarLink corn contains about 13 ppb of Cry9C protein

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[1] The SAP wrote that: "The Agency's analysis results in an upper bound estimate that is considerably high and could be justifiably reduced if several of the issues cited were incorporated. However, this conservative approach results in an estimate with a significant safety factor" (SAP Report page 19). The SAP cited a number of factors that could result in estimated values that are higher than are likely to occur. These factors included: (1) a greater degree of mixing of StarLink and non-StarLink corn than assumed by EPA's high end estimates; (2) the industry practice of preferring corn varieties other than StarLink for producing processed human foods, thus reducing the likelihood that StarLink would be directed to human food channels; and (3) the effects of processing on levels of Cry9C protein in processed food. EPA agrees with the SAP that our November upper bound estimates overstated potential exposure to a considerable degree. For example, the upper bound estimate for mixing in the year 2000 results in about 1.5% StarLink corn. For 1999, the upper bound estimate is about 1.2% StarLink corn, only slightly less than 2000 as discussed in EPA's November Exposure Assessment for the November SAP meeting. These values are three to four times higher than the percentage of the overall U.S. acreage planted to StarLink corn in those years.

per gram of corn starch.

This value for Cry9C protein in corn starch is based on a limited number of analyses by Aventis using the high sensitivity ELISA test from Envirologix. The Agency chose to use this value since it is an actual measured level of Cry9C in corn starch from pure StarLink corn rather than a derived number. The validation of the Envirologix method for detecting Cry9C protein in processed food items in a multi-laboratory study is the subject of a report from FDA.

Using the values discussed above, EPA calculated the amount of Cry9C protein potentially in the diets of adults, infants, and children in the U.S. EPA calculated values separately for each set of “mixing rates.” See Tables 4, 5, and 6 below. Note that these numbers are given in micrograms in order to emphasize the extremely low amounts of Cry9C protein that might be present.

Table 4. Estimated Upper Bound Exposure for Various Population Groups for 2000 Assuming Food Containing Corn Starch Was Made from Grain Containing 1.5% StarLink Corn

Group	Potential Daily Exposure of Cry9C Protein from Corn Starch		
	Upper Bound Exposure for 2000 (1.5%)		
Percentile:	95	99	99.5
US Population	0.00484 ug	0.01379 ug	0.01959 ug
Infants	0.00169 ug	0.00266 ug	0.00290 ug
Children 1 to 6 yrs	0.00145 ug	0.00363 ug	0.00484 ug
Children 7 to 12 yrs	0.00218 ug	0.00605 ug	0.00798 ug

*Data obtained from FDA TAS-DIET Analysis
ug=micrograms

Table 5. Estimated Upper Bound Exposure for Various Population Groups for 1999 Assuming Food Containing Corn Starch Was Made from Grain Containing 1.2% StarLink Corn

Group	Potential Daily Exposure of Cry9C Protein from Corn Starch		
	Upper Bound Exposure for 1999 (1.2%)		
Percentile:	95	99	99.5
US Population	0.00387 ug	0.01103 ug	0.01567 ug
Infants	0.00135 ug	0.00213 ug	0.00232 ug
Children 1 to 6 yrs	0.00116 ug	0.00290 ug	0.00387 ug
Children 7 to 12 yrs	0.00174 ug	0.00484 ug	0.00639 ug

*Data obtained from FDA TAS-DIET Analysis
ug=micrograms

Table 6. Estimated Upper Bound Exposure for Various Population Groups Assuming Food Containing Corn Starch was made from Grain Tested by GIPSA Guidelines Prior to being Channeled to Food Processing and Therefore Contains 0.125% or Less StarLink

Group	Potential Daily Exposure of Cry9C Protein from Corn Starch		
	Upper Bound Exposure (0.125%)		
Percentile:	95	99	99.5
US Population	0.0003249 ug	0.0009261 ug	0.0013161 ug
Infants	0.0001137 ug	0.0001785 ug	0.000195 ug
Children 1 to 6 yrs	0.0000975 ug	0.0002436 ug	0.0003249ug
Children 7 to 12 yrs	0.0001461 ug	0.0004062 ug	0.0005361ug

*Data obtained from FDA TAS-DIET Analysis
ug=micrograms

In summary, EPA believes that the upper bound estimates of potential exposure to Cry9C protein as a result of consumption of corn starch are extremely low. These values range from one-hundreth of a microgram for 1999-2000 estimates to approximately five one-thousandths of a microgram (five nanograms) a day for the estimates using the GIPSA screening protocol.

V. Scientific and Public Comments

Following release of a draft version of this document, an open comment period was held to allow members of the general public to submit letters and/or documents to the Agency regarding the content of this White Paper and corn wet milling. At the same time, several scientists who were members of the November 28, 2000 SAP panel were also asked to comment on the accuracy and relevance of this document as it relates to potential exposure to Cry9C protein in products processed by wet milling.

Four scientists from the November 28, 2000 SAP panel provided comments to the Agency. Each of these scientists indicated that the White Paper was essentially accurate and illustrated the low risk of exposure to Cry9C in food products processed by wet milling. Several of these scientists suggested minor changes to the document. These suggestions were intended to improve either the accuracy or the clarity of the document. All of these changes were considered, and the majority were included in this final version.

Comments that were not incorporated into the document included one in which a commentor (Charles Hurburgh) indicated his concern regarding segregation of StarLink corn to animal feed only as described in Section IV. The Agency agrees that the effectiveness of efforts to segregate StarLink corn to animal feed only were not successful in some cases. However, the estimates in Tables 4 & 5 assume that essentially none of the efforts were successful. Therefore, these estimates would represent the likely maximum potential exposure. This same commentor expressed concerns about the potential presence of StarLink in exported corn gluten. The Agency believes that the focus of this document should be on products intended for use in domestic human food, and not necessarily those fractions intended for animal feed. Therefore, the issue of exported corn gluten is not specifically addressed in this document.

The Agency received public comments from the Corn Refiners Association (CRA), the Nebraska Corn Board, and Environmental Defense. Comments provided by CRA were supportive of the accuracy of the White Paper and its conclusions of no likelihood of health concerns of food products processed by wet milling. Several minor changes to the document were also suggested to provide clarification in the document, and each of these changes was made. The comment from the Nebraska Corn Board was generally in support of establishing a tolerance for StarLink/Cry9C, and did not provide and suggested changes to the document. The comments from Environmental Defense cited concerns about hydrolyzed vegetable proteins, exposure to starch and corn oil.

The concern regarding hydrolyzed vegetable proteins in gluten-derived products made a comparison to hydrolyzed milk formulas which have been reported to produce an immune response. Whereas corn might be a minor part of hydrolyzed vegetable protein, there have been no reports of adverse reactions to hydrolyzed proteins from commodities such as corn (Taylor, Personal Communication, 2001). Although a lack of scientific reports does not insure that there are no potential effects, the Agency believes that this lack of reports, combined with comments from scientific and medical experts, provides a basis for concluding that there is a very low likelihood of adverse reactions to hydrolyzed vegetable proteins.

The second concern regarding exposure to starch suggested the possibility of non-dietary exposures (latex gloves and inhalation in children), a miscalculation concerning soluble proteins, and potential enrichment of Cry9C because of its stability relative to other proteins. The Agency has evaluated data recently submitted by Aventis (MRID# 453866-03) in which various food products made from 100% StarLink were tested for the presence and amount of Cry9C protein. Based upon this information, the Agency believes that there is an extremely low level of protein in corn starch. Of that small amount of protein in corn starch is an even smaller potential amount of Cry9C protein. If corn starch was used as an alternative to talc in latex gloves, there would only be a negligible potential increase in exposure to Cry9C protein as the amount of corn starch on latex gloves would be substantially less than the amount of corn starch consumed in food. Further, since the potential amount of Cry9C which could be inhaled is an even smaller amount, the contribution of the latex glove and inhalation exposure scenarios is expected to be trivial.

The Agency believes, and the scientific experts who reviewed this document agree, that the calculations regarding the potential amount of Cry9C protein in starch do not underestimate potential residual Cry9C. In fact, the November SAP panel indicated that the EPA's high end estimates were "likely to overstate potential exposure" for a variety of reasons as indicated the report from this meeting. The data recently submitted by Aventis looks at levels of Cry9C and total extractable protein (TEP) in food products produced from 100% StarLink, as well as TEP in non-StarLink controls. The values obtained for total extractable protein (TEP) were generally similar between the non-transgenic and StarLink corn products. Finally, with regards to the potential presence of Cry9C protein in corn oil. In general, proteins are extremely rare components of corn oil and the Agency is unaware of any reason to expect a different scenario in corn oil which may be made from StarLink grain.

VI. Recommendation

Based on the above evaluation, EPA believes it is reasonable to conclude that there is virtually no Cry9C protein in food products made from the food fractions of corn produced by wet milling. In addition, 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 to wet milling. Data show that corn protein will not be present in high fructose corn syrup, corn oil, or alcohol (ethanol). Data also indicate that corn starch will contain, at most, such extremely low levels of corn protein that there is virtually no potential human exposure to Cry9C protein from consumption of corn starch.

Continued testing of corn grain for Cry9C protein prior to entry into the food processing chain and diverting any shipments testing positive to domestic animal feed or industrial purposes will insure that food fractions from wet milling contain virtually no Cry9C protein. Such testing will minimize the possible occurrence of shipments of corn containing StarLink from entering the wet milling process.

A possible scenario is for testing whenever a wet milling operation produces food starch. The method would require testing for the presence of Cry9C protein prior to processing using a representative sample of 2400 kernels of corn from selected conveyances (e.g., rail car, barge, truck, etc.) using methods validated by the USDA's Grain Inspection, Packers, and Stockyards Administration (GIPSA) and accepted by FDA. If StarLink corn is determined to be present in the grain, it would have to be diverted to appropriate channels. More details on the methods recommended for dry milling and related assistance can be found at the following web sites:

http://vm.cfsan.fda.gov/~dms/starguid/html
http://www.usda.gov/gipsa/reference-library/handbooks/grain-insp/grbook1/gihbk1.htm
http://www.usda.gov/agency/gipsa/pubs/primer.pdf.

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