The StarLink Affair

A Critique of the Government/Industry Response to Contamination of the Food Supply with StarLink Corn
and
An Examination of the Potential Allergenicity of StarLink's Cry9C Protein

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for
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Key Findings

On Cry9C Protein

* **Cry9C protein in StarLink corn deliberately engineered with a characteristic of food allergens** – elimination of trypsin cleavage site lends lysine mutant Cry9C greater resistance to trypsin digestion (Section 2.2)

* **FDA's allergy test employs surrogate E. coli-produced Cry9C supplied by the petitioner rather than StarLink Cry9C, despite differences in molecular weight and glycosylation** – fails to meet prerequisites for test substance equivalence (Sections 9.2 & 9.3)

* **Allergenicity of some glycoproteins (including food proteins) depends critically on carbohydrate moieties** (Section 9.4)

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* **Aventis and its predecessor companies have failed to characterize StarLink Cry9C:**
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* **Aventis and/or its agents apparently informed farmers that StarLink could be sold for human food use through use of misleading tags on seed bags** (Section 10, Appendix VII)

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* Aventis fails to help supply needed antigenicity data from workers occupationally exposed to Cry9C and animals fed StarLink corn (Section 4.6)

* Aventis breaches standard operating procedure in processing the StarLink corn used to make dry-milled corn product samples tested for Cry9C content, likely resulting in artificially low Cry9C values in products with highest levels of Cry9C; company also fails to explain huge interassay differences in Cry9C measurements (Section 4.4)

Executive Summary

Over the past decade, the scientific and medical communities have become increasingly concerned about the potential of genetically engineered (GE) foods to cause allergies. GE foods produce "novel" proteins that are often new to the human diet and are most often derived from bacteria. Allergies are triggered by aberrant immune system responses, which often occur when a susceptible person is exposed to a new food (or food protein). It is thought that food allergies afflict 2-2.5% of adults and 6-8% of children, or about 8 million Americans. Food allergies are becoming more common, for reasons still unknown. Because GE foods introduce novel proteins, and the process of acquiring allergies is still poorly understood, a growing number of experts recommend labeling of genetically engineered foods and monitoring for potential allergic reactions after market introduction. Mandatory labeling would entail "identity preservation" of genetically engineered crops from field to table. Such a labeling regime would enable doctors to trace possible allergic reactions to GE foods to their source, something that is currently impossible to do in the U.S. Unlike the European Union and at least eight other countries, the U.S. does not require labeling of genetically engineered foods. The identity preservation entailed by labeling also might have prevented or at least mitigated the massive and uncontrolled allergenicity experiment brought on by StarLink contamination of the food supply.

StarLink corn was developed by Plant Genetic Systems (PGS)[1], and contains a genetically engineered insecticidal protein known as Cry9C that is derived from *Bacillus thuringiensis* (Bt), a soil bacterium. StarLink corn was never approved for human food use because of concerns that its Cry9C protein might cause allergies. It was supposed to be used only for animal feed or industrial applications. An expert panel found that Cry9C has a "medium likelihood" of being an allergy-causing protein (allergen) because it exhibits six characteristics of known allergens. PGS increased the potential of Cry9C to induce allergies by engineering it to be more resistant to digestion, a common feature of many allergens. Cry9C also elicits an immunologic response in Brown Norway rats, considered by many to be the best available animal model for predicting the human allergenicity of novel proteins.

Contamination of the grain and food supply has been much more widespread than first thought. Cry9C is now being detected in up to 22% of corn grain tests. Eleven companies have been forced to issue recalls for well over ten million supermarket items and about 2 million lbs. of bulk foodstuffs due to the presence of Cry9C. This contamination has exposed tens of millions of people to some level of Cry9C, which has spread through mixing of StarLink with normal corn as well as spread of the cry9c gene through cross-pollination with normal corn. At least 71 seed companies have reported seed stock tainted with Cry9C. Popcorn, sweet corn, and most recently white corn products have also been contaminated. Millions of people who thought they were avoiding StarLink through choosing white over yellow corn products may have been mistaken. Likewise, companies like Kraft and Taco Bell that switched to white corn will now need to check their products.

Estimates of exposure to Cry9C are fraught with huge uncertainties. No one knows how much StarLink entered the food supply, how many people have consumed Cry9C, or how often, or at what levels. The government has not even developed an estimate of the number of people exposed, focusing instead on level of exposure. The two tests Aventis used to measure the Cry9C content of foods yielded two-fold to nine-fold differences for the very same samples of dry-milled corn products – cornbread, corn muffins, polenta and hush puppies – casting further doubt on the exposure estimates. In addition, irregular procedures in processing the corn used in these tests likely resulted in artificially low values for the Cry9C content of these foods.

[1] Plant Genetic Systems was taken over by AgrEvo, which in turn was acquired by Aventis CropScience.

The government has tested only 17 people who reported suspected allergic reactions to StarLink corn directly to the FDA. Hundreds of corn-related allergy complaints to food companies have been ignored. The FDA has also apparently ignored a recommendation by experts to monitor the medical and allergy communities for additional cases of potential allergic reactions to StarLink from both consumption and occupational exposure. Farm and seed company workers with high levels of occupational exposure to StarLink have been neglected, despite the fact that separate testing conducted in a study funded by the EPA detected immunologic responses to related proteins in farm-workers exposed to Bt sprays. With tens of millions of people exposed to Cry9C, test results from just 17 people cannot form a sound scientific basis for approval of Cry9C residues in foods.

As even the FDA concedes, the allergy tests conducted on 17 people with possible reactions to StarLink could have given false negative results due to use of a surrogate, bacterial-produced version of Cry9C that has different properties than the Cry9C that people were actually exposed to in StarLink corn. Neither Aventis nor the government has characterized these differences, nor determined whether the two versions of Cry9C are similar enough to justify using the surrogate protein for allergy testing purposes. Among other differences, StarLink Cry9C appears to have sugar molecules attached to it, which are added in a process known as glycosylation. The bacterial surrogate protein lacks these sugar molecules. A certain form of glycosylation (N-linked) is often associated with allergenicity. StarLink Cry9C has 5-9 sites suitable for N-linked glycosylation. Other possible differences between surrogate and plant protein have not been (adequately) explored. Finally, the latest report of an allergic reaction by a doctor to a white corn product found to contain StarLink raises further questions about the validity of the FDA's allergy test.

The government's investigation also fails to take adequate account of infants and children, who are more prone to allergies, sensitive to smaller amounts of allergen, and so are at greater risk of allergy to Cry9C than adults. Allergy experts who reviewed StarLink called for special attention to children as the most likely population to acquire allergies if Cry9C were in fact allergenic. They demanded an investigation of the levels of Cry9C in the infant diet, something which the government has not done (beyond a cursory and flawed study of Cry9C in cornstarch).

Infants with food allergies are a particular concern, both because of their greater sensitivity to allergies of all sorts and because they are often placed on hypoallergenic infant formulas rich in corn. Mead Johnson's Enfamil Nutramigen, for instance, contains 54% corn in the form of cornstarch and corn syrup solids. The government has apparently not tested any of these infant formulas for Cry9C, despite the fact that other corn proteins capable of eliciting an immune response are sometimes unexpectedly detected in these formulas. It seems that only one child was included in the Cry9C allergy tests. There has apparently been no outreach to pediatricians to warn them of possibly allergenic corn in the food supply or to collect cases of allergic reactions possibly linked to StarLink. The special risk posed by Cry9C to infants and children argues against granting Aventis' petition to allow Cry9C residues in the food supply.

The StarLink contamination scandal has brought to light many unsavory and hitherto hidden aspects of biotech industry practice and government incompetence in the regulation of genetically engineered foods. Biotech companies regularly submit flawed studies to regulatory agencies, fail to supply crucial data, and flaunt widely accepted testing standards. In perhaps no other regulatory arena are the "competent" government agencies so thoroughly dependent on, subservient to and favorably disposed towards the regulated industry. The FDA and USDA, in particular, have made it their business to promote rather than regulate genetically engineered foods. See Sections 10 and 11 for a further discussion of this topic.

1. Introduction: Allergies, Novel Proteins and the Need for Labeling

The potential of genetically engineered (GE) crops to induce food allergies has been the focus of intense concern over the past decade, as evidenced by the many studies, conferences and workshops devoted to the issue (FDA 1994; Metcalfe et al, 1996; Wal 1998; Consumer & Biotechnology Foundation 1999; Lehrer 1999; SAP II, 2000; EC Scientific Steering Committee 2000; FAO-WHO 2001; to name just a few). It is generally agreed that genetic engineering can increase the allergenic risks of foods in at least three ways:

1) By increasing the levels of allergy-causing proteins (allergens) already found in the plant;
2) By introducing a known allergen into a plant, as happened when a Brazil nut gene was spliced into soybeans, producing soybeans allergenic to Brazil-nut sensitive individuals (Nordlee, 1996); or
3) By introducing a new or "novel" protein from a source organism whose allergenicity is unknown.

This last category raises perhaps the most concern, because the great majority of genetically engineered crops on the market today have been altered with bacterial genes to produce bacterial proteins to which there is little or no history of human dietary exposure. Examples include soybeans engineered for herbicide resistance, by far the most common genetic manipulation, and plants altered to produce insecticidal proteins, such as Bt corn. Bt refers to *Bacillus thuringiensis*, a soil bacterium that naturally produces crystalline (Cry) proteins toxic to certain insects. StarLink is a type of genetically engineered corn that contains a particular Cry protein (Cry9C) which has characteristics of known allergens (i.e. allergy-causing proteins). Some Cry proteins have been used for decades in the form of Bt sprays, and so there is at least some history of dietary exposure to them. Cry9C has apparently never been incorporated into a Bt spray (though there is some dispute about this point), and so represents a new addition to the human diet (EPA Preliminary Evaluation 2000, p. 1).

Food allergies afflict an estimated 2-2.5% of the adult population and 6-8% of children (SAP III, p. 11; Sampson 1999), or about 8 million people in the US alone (based on US Census data). Symptoms range from rashes, hives and swelling to life-threatening anaphylactic shock. An estimated 29,000 episodes of anaphylaxis occur each year in the US, killing an estimated 150 people (Bock et al. 2001). Allergy sufferers can often avoid foods they are allergic to by checking the list of ingredients (e.g. peanut labeling). This is why serious reactions often occur at restaurants and other food service establishments, where meals can be contaminated with allergenic ingredients unbeknownst to the allergy suffer. Genetically engineered (GE) foods pose a similar problem. Without labeling of these novel foods, which requires traceability from field to table, no one can know whether an allergic reaction they have suffered is due to the genetic modification of that food, especially if it comes from a crop like corn that is rarely allergenic. This casts great doubt on the frequent claim that GE foods have not harmed anyone, especially when one recalls the similar claims once made for numerous chemicals now known to be toxic, such as asbestos, PCBs and dioxins. Then as now, "don't look, don't find" is not a very convincing scientific protocol. In part from concern over the rising incidence of food allergy (Wal 1998, p. 413; SAP I, p. 12), the causes of which are still obscure, an increasing number of scientists have recommended consideration of post-marketing surveillance of GE foods (Wal

1998 & 2001; SAP I, p. 11; SAP II, p. 11; Consumer & Biotechnology Foundation 1999, section 5.2; FAO-WHO 2001, p. 9).

Supporters of labeling and post-market surveillance of GE foods believe that the huge gaps in our knowledge of food allergy, coupled with the immunologic uncertainties of novel, genetically engineered proteins in the food supply, warrant such a precautionary approach. As allergists readily admit, it is extremely difficult to make valid generalizations in this field. Some prior exposure to the allergen is necessary for "sensitization" (acquiring the allergy), but how much exposure and for how long varies greatly depending on the allergen, the person, age and frequency of exposure, and many other factors (SAP I, p. 10). In some cases, exposure to billionths of a gram is sufficient to induce an allergy or allergic reaction (Businco et al, 1999). Even a single exposure could possibly sensitize the immune system (Dr. Ricki Helm, SAP Transcript, p. 446). There are reports of infants becoming sensitized through breast milk and fetuses acquiring allergies *in utero* (SAP III, p. 16). Some people may even become allergic to a food through inhalation of trace quantities (FDA 1994, pp. 219-20; Urisu 2001, p. 7). Many allergens are common components of the foods in which they are found; yet proteins present in "infinitesimally small quantities" (e.g. in soybeans) can also be allergens (FDA 1994, p. 145). The only thing that everyone seems to agree upon is that children, especially infants, are at the greatest risk of allergic sensitization to novel, genetically engineered proteins (see Section 8).

2. Is Cry9C an allergen?

2.1 Cry9C Possesses Six Properties of Food Allergens

According to the StarLink Scientific Advisory Panel that met on November 28, 2000 (SAP III), there is a "medium likelihood" that Cry9C is an allergen. This assessment was based on the fact that Cry9C possesses six properties characteristic of allergens (SAP III, p. 10):

1) Possible presence in rat blood after oral feeding;
2) Induction of an immunologic response in rats;
3) A molecular weight in the range typical for allergens;
4) Resistance to breakdown by acid;
5) Resistance to digestion by proteases (enzymes that break down proteins); and
6) Its probable status as a glycoprotein (discussed in Section 9)

Cry9C is also heat-resistant (Noteborn 1998; Peferoen 1997b), another characteristic of many food allergens (Sampson 1999) that impacts the protein's ability to survive food processing intact.

2.2 Lysine Mutant Cry9C in StarLink Engineered for Resistance to Digestion

The Cry9C insecticidal toxin produced by StarLink corn differs substantially from the native Cry9C expressed in bacteria. The bacterial Cry9C protein is about twice as large (130 kD), and has no toxicity to insects until the toxic fragment (≅ 70 kD) is cleaved from it, a process that occurs in the guts of certain insects such as the European corn borer. To produce StarLink, scientists spliced the shortened gene sequence encoding the toxic fragment, but with a slight modification, into corn. This modification involved mutation of the DNA sequence for a single amino acid, changing the naturally occurring arginine at position 164 to lysine. This single amino acid alteration made the lysine mutant Cry9C protein more resistant to breakdown by a

pancreatic digestive enzyme (trypsin) than the naturally occurring bacterial protein. The intent of this alteration was to make Cry9C more toxic to insects by inhibiting its breakdown to a non-toxic, 55 kD fragment through elimination of a trypsin cleavage site (Lambert et al 1996, pp. 84-85). Resistance to digestion is a characteristic of most food allergen proteins, as noted by many scientists (e.g. Helm 2001, pp. 2-3) and SAP III (No. 5 above). Thus, StarLink's developer intentionally engineered Cry9C with a trait that is more likely to make it allergenic, with the goal of increasing its effectiveness as an insecticide (AgrEvo Safety Assessment 1998, p. 49).

2.3 Digestive Stability May Explain Other Allergenic Properties of Cry9C

Trypsin is a pancreatic enzyme released into the small intestine. In addition to its partial trypsin resistance, experiments have shown that Cry9C is resistant to pepsin, a stomach enzyme (Noteborn 1998). Resistance to these two digestive enzymes may help explain two other allergenic properties of StarLink's Cry9C (Nos. 1 & 2 above).

Dr. Hubert Noteborn, an expert in the field of Cry proteins, detected low levels of Cry9C in the blood of rats after feeding them high doses of the protein orally. He states that Cry9C probably survives "to be absorbed via the intestinal mucosa during consumption" and may then "trigger the production of antibodies, including the antigen-specific immunoglobulin E [IgE] antibodies" that are associated with allergies (Noteborn 1998, p. 22).

This finding was corroborated in a second study in which oral administration of either Cry9C protein or Cry9C corn extract was found to induce an immunologic (IgE) response in Brown Norway rats (BIBRA 1998). And while this result does not by any means prove that Cry9C causes allergies in humans, many allergists believe that this particular strain of rat represents the most promising experimental animal for use in predicting the allergenic potential of novel food proteins such as Cry9C, in part because Brown Norway rats react to many of the same proteins[2] that cause allergies in humans (Penninks 2001; Atkinson 1994).

Both Aventis and its predecessor company, AgrEvo, criticized this study in part because the rat is not a validated model for allergy testing purposes. Yet there is no such animal at the present time, and allergists understand that it provides suggestive, rather than conclusive, evidence of a protein's allergenic potential. AgrEvo also pointed out that the study was flawed due to the use of control corn that was contaminated with Cry9C[3]. Contamination of the control corn prevented definitive conclusions from being drawn concerning Cry9C's allergenicity. However, nowhere in this critique is it acknowledged that this contaminated control corn, along with the other test substances, was supplied to the firm that conducted the study by Plant Genetic Systems, the original developer of StarLink.[4]

[2] For example, allergenic milk and chicken egg proteins.

[3] "Control corn" refers to non-genetically modified corn. It was used in the experiment for the purpose of comparing the immunologic response of rats fed normal corn to that of rats fed genetically engineered corn or purified Cry9C protein.

[4] Aventis has full access to the records of both of its predecessor companies.

3. Cry9C Contamination of the Food Supply Spreads

3.1 StarLink in the Grain Supply

Although StarLink corn was grown on only a small percentage of U.S. cornfields over the past three years, contamination has been widespread. Despite the efforts of the USDA and Aventis to contain the spread of StarLink, Cry9C is being detected throughout the grain supply. Aventis reported the presence of Cry9C in 430 million bushels of corn (Washington Post, 3/18/01), much more than previously assumed. More recently, 9% of 110,000 grain tests conducted by the USDA since November 15, 2000 at sites across the country turned up positive for Cry9C. More sensitive tests carried out since February 2001 revealed a 22% rate of contamination (Boston Globe, 5/3 & 5/17/01). Much of this contamination appears to be due to mixing of StarLink grown in 1999 with other corn, before containment measures were begun.

Yet the discovery of Cry9C in popcorn and sweet corn (Lincoln Journal Star, 3/29/01) as well as in seed corn clearly indicates that StarLink has cross-pollinated with other varieties, spreading its cry9c gene through wind-blown pollen. The contamination of seed corn is extensive and growing. Since November 21, 2000, when Garst Seed Company announced that one of its non-StarLink hybrids was found to contain the cry9c gene, over 70 seed companies have reported the same problem. The USDA is buying back nearly 450,000 units of non-StarLink, Cry9C-contaminated seed corn from 71 of 288 seed companies that USDA contacted. Tainted seed dates anywhere from production year 1997 to 2001. With one unit equal to a bag of 88,000 kernels, this represents nearly 40 billion non-StarLink kernels tainted with Cry9C (USDA News Release). Popcorn, sweet corn and seed stock growers take special care to maintain the purity of their lines. If even their corn is tainted with Cry9C, contamination has certainly not been contained.

3.2 Cry9C Corn in Processed Foods

From September 22, 2000 to March 29, 2001, at least 11 companies have initiated recalls of corn products due to contamination with StarLink (see Appendix I). Affected products include taco shells, tortillas, tortilla chips, enchiladas, cornmeal, corn flour, dry soup mixes, brewing flakes for beer-making, and veggie corn dogs. Mission Foods, one of the largest suppliers of Mexican foods in America, recalled 297 products under 87 brand names, many of them the in-house brands of supermarkets such as Safeway, Albertson's, Best Buy, Food Lion, Kroger and Shaws. Some of America's best-known companies were implicated, including Kraft Foods (Taco Bell taco shells), Campbell's (taco shells) and Kellogg's (Morningstar Farms and Loma Linda veggie corn dogs). Bulk foodstuffs were also found contaminated with StarLink, forcing ConAgra to recall nearly 1.5 million lbs. of cornmeal, corn flour, polenta grits and similar products. Items subject to recall were distributed all over the country, as well as Canada, Japan, Korea and the Caribbean. As discussed below, the FDA has probably tested only a small fraction of corn product lots that made their way to supermarket shelves, so the true amount of contaminated food is surely many times higher.

Because StarLink is a yellow corn, many food manufacturers have shifted to white corn to avoid StarLink contamination. This strategy may no longer be effective with the recent discovery by the FDA that StarLink contamination has spread to white corn products (FDA Letter, June 21, 2001). Dr. Keith Finger, one of the participants in the CDC/FDA allergy testing

program, has reported a suspected allergic reaction to white corn tortilla chips that contain StarLink DNA.

If the past is any indication, StarLink and its Cry9C will continue to turn up in ever more grain and processed foods. Volunteer growth and cross-pollination almost assures that the problem will extend into the future. Given these facts, it is difficult to accept the assurances of Aventis that Cry9C has been contained. If it were, the company would not need to seek a tolerance. Most importantly, the spreading contamination casts serious doubt on the exposure estimates made thus far (SAP III, p. 21).

4. Exposure to Cry9C Corn: How Many, How Frequent, How Much

The issue of exposure to Cry9C involves at least four questions: 1) How many people have been exposed? 2) How frequently have they been exposed? 3) How much have they been exposed to? and 4) How accurate are the measurements of Cry9C in foods?

4.1 How much?

Aventis and the government agencies have focused almost exclusively on the third question. While estimating the level of exposure to Cry9C is important, the exclusive emphasis on this one parameter seems misplaced for several reasons. First, since even infinitesimal quantities of an allergen can sensitize and cause reactions, no level of exposure can be considered safe. And since even denatured or fragmented protein can be allergenic, food processing might not reduce the allergenicity of Cry9C (assuming it is an allergen). In fact, denaturation may even produce new epitopes (allergy-inducing components), and so processing might actually transform Cry9C into a (more) allergenic protein (SAP III, pp. 13-14). Secondly, the estimates of exposure differ so widely as to be little more than "speculation" (SAP III, p. 26). The EPA's upper-bound exposure estimate was seven-fold greater than Aventis' (EPA Preliminary Evaluation 2000, p. 14). The Scientific Advisory Panel proposed an "absolute worst-case exposure scenario" which, though highly unlikely, suggests an estimated exposure "two orders of magnitude [100-fold] higher than the Agency's current upper bound estimate" (SAP III, p. 20). Thus, estimates of exposure vary by a factor of 700. Thirdly, as noted above, no one has even attempted to take into account the potential additional exposure due to the seemingly uncontrolled spread of the cry9c gene to other varieties of corn. Finally, there is the possibility of still more exposure to closely related Cry9 protein(s) that may be found in certain Bt sprays (Aventis Updated Safety Assessment 2000, p. 10). If actually present, these related proteins might share epitopes with Cry9C, and so could play a role in sensitization to the protein. This factor has also gone unaccounted for.

Given these many levels of uncertainty, it is no wonder that Panel member Dr. Macintosh expressed frustration at being asked to draw conclusions from such deficient data.

> **"Therefore, my conclusion to this second question, how meaningful are the upper bound estimates, is that it's not very meaningful at all. I don't see that there's anything to do with this exposure number except look at it and say is it good or bad. There's nothing else to do with it, it's not very meaningful."** (SAP Transcript, p. 544)

4.2 How many?

The fundamental question of how many people have been exposed to Cry9C corn has barely even been addressed. For instance, the EPA could do no better than establish a range of 1% to 40% for the amount of StarLink grown in 1999 that was ***not*** directed to animal feed or industrial uses and, thus, could have been misdirected into the human food supply chain (SAP III, p. 20). If the true figure is closer to 40%, many more people will have been exposed than if it is 1%. There is also considerable uncertainty about how uniformly Cry9C corn was mixed with other corn in the grain handling system. With more uniform mixing, as assumed by Aventis, more people will have been exposed to lower levels, while the less uniform mixing proposed by the EPA results in fewer people exposed to higher levels of Cry9C corn (EPA Preliminary Evaluation, p. 17-19). It is true that the data available to answer the question "how many" are extremely poor; yet they are certainly no worse than the numbers on exposure levels, and there have been reams of analysis devoted to this parameter. As suggested above, **the inability to establish a safe level of exposure to Cry9C would seem to make an estimate of the number of people exposed to any level at least as important as level of exposure**. Therefore, it is puzzling and disappointing to discover that the government has not tackled this crucial question (personal communications, William Jordan & Janet Andersen, EPA).

We might gain an admittedly rough idea of this important parameter by considering the quantity of supermarket products and bulk foods found to be contaminated (Appendix I). Based on data from seven FDA Enforcement Reports, over 1,000,000 ***cases*** and 700,000 items of supermarket products were subject to recall. Due to lack of data, these figures exclude what is certainly the largest single recall, that of Mission Foods, which involved 297 products under 87 brand names. A typical "item" is a box of taco shells or a bag of tortillas. The number of items per case varies, and is not always cited in the FDA's data. If one conservatively assumes 10 items in each case, then 11.5 million items were subject to recall. The true figure is much higher – perhaps several times higher – due to the exclusion of Mission Food products. Bulk foodstuffs subject to recall include corn flour, cornmeal, bulk dry soup mixes and beer-brewing flakes. The four companies for which data are available issued recalls for nearly 1.8 million lbs. of their products. Figures are unavailable for two producers of bulk foods, so this figure is also an underestimate.

Because there are no publicly available data on the proportions of supermarket products and bulk foods that were in fact retrieved before sale or withdrawn before processing, respectively, it is difficult to estimate the quantity of Cry9C-tainted food that was actually consumed. A rough estimate could certainly be developed, though neither the FDA nor the EPA appears to have done so. This factor would reduce the estimates of 11.5 million items and 1.8 million lbs. cited above, perhaps by half or more.

On the other hand, one must consider the extent of the FDA's Cry9C food testing regime. Given the agency's limited resources, it seems highly likely that most lots of numerous corn-containing products went untested. This factor could be accounted for by estimating the ratio of the number of corn-containing product lots tested to the total number of corn-containing lots. One would presume that roughly the same proportion of untested products as tested items would turn up positive in any given time frame, assuming representative sampling. This adjustment would greatly increase the estimated number of contaminated products actually consumed, perhaps by one or two orders of magnitude (10 to 100 times) or more.

Taking both factors into consideration, **a conservative estimate of the number of consumed items tainted with Cry9C is in the tens of million**. Since each item (e.g. bag of corn flour or tortillas, box of taco shells) is often consumed by several people, say members of a

family, the number of exposed people is several times higher than the number of tainted items. With appropriate data, the method outlined here could be used to develop at least a rough estimate to address the fundamental question of how many people have been exposed to Cry9C. Panel member Dr. Dean Metcalfe spoke loosely of an "exposed population which could be a million people…" (SAP Transcript, p. 558). As we have seen, this estimate is almost certainly much too low in light of the expanded contamination and additional food recalls that have come to light since the SAP meeting in November of 2000.

4.3 How frequently?

Another important factor that has not been investigated is how frequently consumers have eaten StarLink and other corn containing Cry9C. According to William Jordan of the EPA, approximately 80 percent of the U.S. population eat some food that contains corn protein on any given day (SAP Transcript, pp. 99-100). Panel member Dr. Macintosh asked the EPA's Jordan this question at the SAP III hearing.

> "The analysis, the exposure assessment done here, is for a single day for a single person. Does – in what way does this assessment support analyses of repeated intermittent exposure over multiple days or months for a person?"

Mr. Jordan's response:

> "…the material that we've provided in writing does not shed much light on the frequency of encountering – the possible frequency of encountering a Cry9C protein residue in food that people eat." (SAP Transcript, p. 99)

4.4 How accurate are the measurements of Cry9C in foods?

In addition to the uncertainties cited above, there are serious questions about the accuracy of the assays currently being used to test for levels of contamination. The method used to measure Cry9C in foods is based on the use of antibodies specific to Cry9C protein. It depends on the capacity of these antibodies to "capture" or bind all of the Cry9C protein (and no others) in extracts of food products. In Section 9, the uncertainties associated with this immunological detection method are discussed with respect to the closely related allergy-testing assay, which measures Cry9C antibodies in blood. In essence, the food assay uses antibody to capture Cry9C protein, while the allergy test employs Cry9C protein to capture its specific antibody. Flaws in the allergy test, which would apply equally to the food test, are discussed in Section 9.

Apart from this issue, the SAP suggested that Cry9C levels in food may be underestimated due to the failure to detect Cry9C fragments and/or denatured protein resulting from food processing. Fragmented or denatured Cry9C may also be allergenic (see Section 4.1). Another problem cited by the SAP is Aventis' use of short extraction times for food samples, which might also result in underestimated protein levels (SAP III, p. 15).

Still another troubling indication of the unreliability of Cry9C tests comes from the latest submission by Aventis (Aventis Detection 2001), in which a full range of StarLink corn products and fractions were tested. It should be mentioned that these assays are supposed to be much improved over prior tests thanks to the use of polyclonal rather than monoclonal antibodies. Despite this supposed improvement, **Aventis discovered that two variants of the same basic assay yielded results that differed *by two-fold to nine-fold* on the very same samples of dry-milled corn products** (see Appendix II). Both variants were based on polyclonal antibodies,

one developed in-house by Aventis and the other a commercial assay. The corn products tested here apparently had relatively high levels of Cry9C protein (up to several micrograms per gram sample): corn bread, corn muffins, polenta and hush puppies. Aventis claims that the differences between the two assays "reflect differences in the extraction buffers and methods used" (Aventis Detection 2001, p. 27). Yet the company offers no evidence to support this assertion, and it is impossible to judge the matter because the composition of the commercial assay buffer is a "trade secret" (Ibid, p. 24), and extraction times are not specified for either assay. (Aventis includes instructions for the commercial assay which call for extraction times of 3 hours to overnight, but the time(s) actually used are not stated.) The failure to specify extraction times is particularly disappointing given the SAP's explicit criticism of the short extraction times used in prior tests conducted by Aventis, which decreases the amount of Cry9C detected.

Close inspection of this study reveals several other irregularities. To take one of several examples: In processing the StarLink corn that was used to make the dry-milled corn product samples tested for Cry9C content, Aventis dried the cornstock at temperatures of 130-160° F for 120-124 minutes (Ibid, p. 103) rather than for 30 minutes, as prescribed by standard operating procedure (Ibid, p. 99). There is no explanation given for this four-fold deviation from SOP. Although Cry9C is relatively resistant to breakdown by heat, the excessively long heating at these high temperatures would likely denature more of the protein, and perhaps render it undetectable. In any case, the processing should have been repeated with a new batch of corn heated for the proper length of time. It is interesting to note that this particular batch of corn was used to make dry-milled corn products, which as discussed above showed relatively high levels of Cry9C. The proper (shorter) heating time would likely have resulted in still higher levels.

4.5 From "Speculation" on Exposure to "Hard Evidence"

Aventis has generated reams of analysis, particularly in its latest submissions, to support its claim that even if Cry9C is an allergen, it is of no public health concern because exposure levels are supposedly too low to sensitize or elicit reactions. As detailed above, **the huge uncertainties associated with estimates of exposure to Cry9C undermine any exposure-based argument presuming to show the "safety" of Cry9C in the food supply.** And it was precisely due to frustration with such poor exposure data that the Scientific Advisory Panel labeled these efforts as "speculation" and stressed the importance of investigating allergy reports instead:

> **"Given the current state of knowledge regarding allergens and the uncertainties of ascertaining the exact amounts of Cry9C in the food chain, this approach [antibody testing] could provide "hard evidence" as opposed to *speculation* on the question at hand."** (SAP III, p. 26, emphasis added)

Panel member Dr. Marc Rothenberg underscored this point:

> **"…in my opinion then there's a potential great underestimate of the protein out there that could be triggering an immunological response."** (SAP Transcript, p. 86)

Asked to prioritize the most important information needed to better assess Cry9C's potential to cause allergies, SAP III's top two items were:

1) **Testing people with alleged reactions to StarLink corn for antibodies to Cry9C**; and

2) **Monitoring for reports of additional allergic reactions possibly linked to consumption of or occupational exposure to StarLink.** (SAP III, p. 26)

4.6 Aventis ignores past SAP recommendations

If Aventis had followed the recommendations of past Scientific Advisory Panels, we would now have much more data on which to judge the question of Cry9C's allergenicity. But Aventis has demonstrated a pattern of ignoring the Panel's experts. Thus, SAP III found the studies submitted by Aventis in the Fall of 2000 to be of little use:

> **"…no new data were presented which provided any convincing evidence that Cry9C potential allergenicity was reduced."** (SAP III, p. 10)
>
> **"The Panel members were uncomfortable with the available data; there was an expectation of more antigenicity/allergenicity data based upon prior SAP meeting discussions and recommendations."** (SAP III, p. 12)

The reference here is to the first Scientific Advisory Panel (SAP I), which met on February 29, 2000 to advise the EPA on Cry9C's potential allergenicity to help the Agency decide whether to approve StarLink for human food use. This was over six months before the first report of contamination. SAP I called on Aventis to help provide immunological data through the following actions:

1) Collection of sera from animals fed StarLink to test for antibodies to Cry9C;
2) Collection of sera from humans exposed to StarLink pollen for antibody testing (sensitization to Cry9C could occur through inhalation of pollen). In fact, **the SAP specifically recommended setting up a monitoring program for agricultural workers at Garst Seed Company, the largest seller of StarLink, for this purpose** (SAP I, pp. 8-9). ***These data have still not been collected.***

Such testing can and should still be carried out in order to supplement the meager data obtained from the small group that recently underwent testing for antibodies to Cry9C.

5. FDA/CDC Investigate a "Handful" of Allergy Reports

5.1 Limited Scope of the FDA/CDC investigation

The Food and Drug Administration (FDA) and Centers for Disease Control (CDC) have collaborated to investigate reports from consumers who reported to the FDA what they believe to be allergic reactions to StarLink corn. Of the 51 reports received by the FDA, the CDC considered 28 to be likely allergic reactions. Of this group, the 17 who filled out questionnaires and volunteered sera were tested for the presence of Cry9C antibodies. The investigated reactions occurred in the period from July 1 through November 30, 2000 (CDC Investigation 2001, pp. 5-6). The group that was tested includes at least two people who apparently experienced anaphylactic shock.

1) **Grace Booth** began to experience difficulty breathing (her throat constricted) just 15 minutes after eating a lunch of chicken enchiladas; she lost her voice, her lips swelled, and her body

itched all over. Paramedics were called and administered several shots of Benadryl and put her on an IV. Tests for all other food allergies came up negative.

2) **Keith Finger** experienced a stomachache and diarrhea shortly after eating a dinner of tortillas, beans and rice. Shortly after, he started to itch all over, his tongue began to swell and he had difficulty breathing – all the symptoms of anaphylactic shock. He treated himself with 300 mg Benadryl, then with epipen 15 minutes later. He is convinced he would have died without this self-treatment (FDA Consumer Complaint/Injury Report forms SAN 2578 and FLA 0470, respectively).

5.2 Allergy complaints since November 30, 2000

At least 29 individuals have contacted the FDA or EPA with suspected allergic reactions to StarLink corn since the cutoff date of November 30, 2000 (FDA Evaluation 2001, Figure 1). Appendix III summarizes 12 suspected allergic reactions to StarLink with report dates ranging from 11/30/00 to 1/8/01. It is not clear why those who reported reactions in December of 2000 (15) and January of 2001 (3) have been excluded from the investigation. At this time, the ELISA sera testing assay was still under development. Since the SAP has stated that more cases would provide additional support for proving or refuting the allergenicity of Cry9C, and the number of cases investigated so far is extremely small, the FDA should expand the investigation to include these as well as more recent complainants (11 complaints received in March and April 2001). Finally, Dr. Keith Finger recently reported another allergic reaction to a white corn product shown to contain StarLink. He should be reexamined, perhaps with a more reliable allergy test.

5.3 One report of a death allegedly linked to Taco Bell restaurant food products

There is also one report of a death submitted to the EPA StarLink docket and the FDA by private investigator Chuck O'Neill of Investigation Associates. According to this letter, a Mr. Buddle "experienced immediate respiratory failure after ingesting two taco products purchased from Taco Bell on April 11, 2000. Within a very short period of time, Mr. Buddle experienced severe respiratory difficulty which resulted in cardiac arrest ultimately causing his death on April 30, 2000." "There is preliminary indication that Mr. Buddle's condition was caused by ingestion of an inferior food product from Taco Bell." The letter requests a full investigation of the matter by the United States government. It is unclear whether there has been any follow-up on this case.

5.4 FDA's passive "self-reporting" system

Speaking of the FDA's adverse event reporting system, Dr. Karl Klontz states that:

> **"This surveillance system is really a passive*, and I underline passive report system,* because these are spontaneous reports that are sent to the Food and Drug Administration…"** (SAP Transcript, p. 110)

We would do well to consider the limitations of such a passive report system. "Passive" here means that the FDA made no systematic attempt to alert health professionals or the public to the potential health threat posed by Cry9C corn. Instead, both physicians and the public were (and are) left to learn what they might haphazardly, from media accounts of StarLink contamination and food recalls. Alerted to the possibility of allergenic corn, physicians might well have

discovered and reported to the FDA cases of possible reactions to StarLink in their patients. With proper guidance, allergy support groups could have done likewise. This would have resulted in a larger pool of potentially affected people for further investigation, which could only increase the meaningfulness of results from the allergy testing program.

For a different perspective on the same problem, imagine for a moment that Cry9C is in fact allergenic and that you, an average American, have had an allergic reaction to it. What are the chances that you will be able to trace that reaction back to Cry9C corn? In order for a report of your reaction to reach the FDA through its passive report system, you would have to: 1) Single out corn – a food very few people associate with allergies – as a possible cause of your reaction, and do so without informed guidance from your physician; 2) Have been alerted to StarLink through the media; 3) Remain unconvinced by the many official assurances of little or no risk; 4) Know that the Food and Drug Administration accepts reports on adverse events to foods; 5) Be confident enough to actually call the Agency and report your complaint.

If you fail to pass any of these screens, your complaint will go unregistered. The latter screens are particularly important. Many people have little contact with federal agencies in their daily lives and are intimidated by the prospect of calling up the government. It is especially significant that the population segment with the highest dietary exposure to corn, and hence StarLink, is the Hispanic community. Hispanics comprise a disproportionate percentage of agricultural workers, some of whom will be exposed to high levels of (Cry9C) corn through inhalation of pollen or corn dust as well as in their diet. IgE antibody formation to Bt sprays has already been demonstrated in agricultural workers, presumably elicited via dermal and inhalational routes (Bernstein et al., 1999). As noted above, SAP I was concerned about inhalational sensitization to StarLink, and explicitly recommended setting up an allergy monitoring program for Garst Seed Company workers. SAP III Panel member Dr. Hubert Noteborn, speaking generally of food allergens, concurs: **"… it is unknown whether sensitization takes place through the intestinal tract or through inhalation *or even both*."** (SAP Transcript, p. 421, my italics)

And what if you belonged to the group with the highest risk of all – children? Young children cannot "self-report," and instead must depend on their parents to interpret whether they have suffered an allergic reaction. Children generally consume greater amounts of corn than adults, particularly food-allergic children on special diets (see Section 8). Yet there appear to be surprisingly few children in the allergic complaint reports obtained thus far. While this could be a welcome sign that children have not been affected, it is more likely a troubling indication of the deficiencies of a passive "self-reporting" system, especially when one considers that children are 3-4 times more prone to allergy than adults. One wonders how many more children might have been reported had the FDA taken a precautionary approach and alerted pediatricians to the possibility of allergic reactions to corn products as soon as the contamination was first discovered.

Finally, one must compare the number of cases investigated (51) and the number tested for Cry9C antibodies (17) to the size of the exposed population. If as indicated above tens of millions of people have consumed products containing StarLink corn, it would be difficult to accept the results of an investigation involving 50 reports as in any way representative of the exposed and potentially affected population.

For all of these reasons, it seems likely that substantial numbers of people with potentially allergic reactions to Cry9C corn have not reported their complaints to the FDA and thus are not being investigated.

5.5 Aventis and the food industry attempt to downplay allergy reports

With such an inadequate reporting system delivering so few complaints from such a large exposed population, it is both surprising and disappointing to hear Aventis and the food industry continually downplay the significance of the few allergy reports that have been investigated. The following argument is repeated again and again: Since the number of allergy reports has tended to increase (sometimes dramatically) after publicity over a Cry9C corn recall, these reports are somehow less credible, "involving, for example, the tendency of individuals to associate an illness (the flu, for example) with information about a product that they have learned from the news media" (GMA Submission 2000, section II).

This argument, however, ignores an obvious fact. Corn is a rare food allergen. Most people would not single it out from other foods they had eaten as a possible cause of an allergic reaction they had suffered. Thus, before the StarLink scandal broke, one would not expect many reports of allergies *even if Cry9C is in fact an allergen.* In other words, it is possible that reactions linked to Cry9C corn did in fact occur in the months and years before September 18th, 2000, but that people failed to report them from ignorance of the possibility that the corn they were eating had caused them. And while it is certainly true that some people will mistakenly identify reactions they have suffered as allergic, when in fact they involve some other illness, such reports can be eliminated from consideration without too much trouble. This is precisely how the CDC/FDA proceeded in their investigation – by eliminating complaints describing symptoms that are not typical of allergies.

If the purpose of the investigation is in fact to protect public health and not limit a company's liability, it is far better to collect a larger group of potentially relevant reports, whittle those down to complaints representing true allergic reactions, and test this select group, rather than attempt to draw conclusions from a group much too small to be representative.

One of the nation's leading allergists, Panel member Dr. Hugh Sampson, sums it up best:

> **"It was suggested that until the first reports of the Cry9C came out in the press, nobody really reported adverse reactions to corn. That to me is not surprising. I think that the majority of people don't in any way suspect corn as being a major allergen and would have no reason to suspect that any kind of adverse reaction associated with a meal in which they ingested corn would provide a problem. So, I don't think the fact that nothing came up before that should be considered as a reason to believe that these reactions are not, in fact, real."** (SAP Transcript, p 461)

6. Hundreds of allergy reports go uninvestigated by FDA/CDC

On October 30th, 2000, the FDA, EPA and USDA sent a joint letter to four food industry trade groups – Grocery Manufacturers of America (GMA), National Food Processors Association (NFPA), Food Marketing Institute and Snack Food Association – requesting their assistance in obtaining information from member companies on any allergic reaction complaints

the companies had received that could be related to StarLink corn.[5] The discussion below is based on responses from GMA and NFPA (see Appendix IV for a fuller discussion of these submissions). We are not aware of any responses from the Food Marketing Institute or Snack Food Association.

6.1 Grocery Manufacturers of America

According to its submission to FDA, the GMA received information from 9 major food companies. GMA then provided the information to its legal counsel, who in turn passed it on to an unidentified firm (or individual, the name is blacked out), which analyzed and aggregated the data. The GMA then responded to the agencies with a submission containing summary responses (sometimes a single sentence) and/or limited statistical data for each of the 9 companies. The companies are not identified by name.

While GMA claims that "there has been no increase in reports to food companies … concerning potential allergic reactions to foods that contain corn (and which may have contained Starlink corn) over the last several years," data from at least two of the companies directly contradict this statement.

* In the 6-week period from 9/18/00 to 10/30/00, "Company Three" received 28 allergic complaints. **This represents a 226% increase in the frequency of allergic complaints relative to the preceding 2 ¾ years**. These figures pertain to consumer complaints for all foods, not just those which contain corn.

* "Company Seven" data refer specifically to alleged allergic reactions to products that contain "yellow corn/corn meal." Complaints are reported as a ratio of "complaints/MM lbs. product." **The frequency of complaints per pound of product in the period from 9/17/00 to 10/28/00 rose by a full 277% over the average for the preceding period** (1/1/98 to 9/16/00). GMA does not report the actual number of allergic complaints received by Company Seven for any period.

Information provided for the other seven companies is of even lesser quality. For three companies, no statistical health data are supplied at all, but rather only one to two-sentence responses. For instance:

* The full response reported for Company Nine is: "[W]e have not had a single consumer call claiming to have an allergy to Cry9 protein." [sic]

This response is clearly inadequate, since it might mean that complaints in which persons mentioned "StarLink corn" or corn products in general (versus Cry9 protein) were excluded.

[5] The letter stated that the groups need not submit information on alleged complaints if the only corn ingredient the relevant product contained was corn syrup, corn starch, ethanol or corn oil that is not cold pressed. Since such products are less likely to contain substantial levels of protein (and so Cry9C), the complaints reported below likely involve corn products with higher levels of protein (including Cry9C).

6.2 National Food Processors Association

The NFPA's submission is likewise scanty, and does not even break down the allergic complaints by company, but rather aggregates data received from 11 companies into a single table. According to the submission:

* There were 31,580 allergy/health-related consumer contacts with these 11 companies in the 2-month period from 9/18/00 to 11/17/00. **The frequency of consumer allergy/health contacts in this 2-month period rose over 12 times relative to the preceding 2 ¾ years.**

* Of these 31,580 contacts, ***210 reports involved allergy complaints in which yellow-corn containing products were specifically mentioned***. ***Yellow corn-related allergy complaints were 90 times more frequent in this 2-month period*** relative to the preceding 2 ¾ years.

* Of these 210 people who claimed allergic reactions to yellow corn products:
  + 53 (25%) spoke with a company-retained physician, who "found no confirmed cases of allergic reaction."
  + ***74 (35%) "sought medical treatment with a physician"***
  + ***20 (10%) "sought medical attention in an emergency room"***
  + 72 (34%) had no contact with a physician.
  + 1 consumer had a sudden onset of rheumatoid arthritis, which her doctor believes may be from GE ingredients

6.3 FDA Should Conduct a Full Investigation of Allergy Reports Received by Food Companies

Given the SAP III's insistence on the need to evaluate additional allergic reaction reports (see Section 7), it seems clear that FDA should thoroughly investigate all of the unsatisfactory summary data provided by GMA and NFPA. The agencies requested that food company reports be sent directly to FDA rather than filtered through trade association lawyers. They also requested full information – which would include basic data such as name of complainant, address, telephone number and description of symptoms – rather than largely useless statistics. Concern for liability on the part of the food industry must not be allowed obstruct investigation of a public health threat. Therefore, the FDA is urged to:

1) Obtain from NFPA member companies as much information as possible on the 210 allergic complaints linked to yellow corn products, subject these reports to the same analysis accorded the FDA complaints, and test the sera of any of these individuals (assuming they consent) who might have suffered true allergic reactions. Highest priority should go to the 94 people who sought medical attention, but it is clearly necessary to review the other cases as well, especially the 53 people who spoke with a company-retained physician, given the obvious conflict of interest involved here.

2) Conduct a similar investigation of complaints received by the companies reported in the GMA submission. These individuals should also be included in the FDA/CDC's allergy testing program, as appropriate.

3) Set up a monitoring system to route all present and future corn-related health complaints from food companies to the FDA for further investigation.

7. Scientific Advisory Panel Calls for Expanded Investigation

At the SAP III hearing, Dr. Carol Rubin of the Centers for Disease Control stated that: **"…all we're dealing with really is a handful of self-reported cases right now…"** (SAP Transcript, pp. 135-36). In recognition of the limited population being investigated, the Scientific Advisory Panel recommended surveillance for additional reports from "individuals who claim to have experienced adverse effects either after consuming food that might have been made from StarLink corn *or from occupational exposure to StarLink corn*." (SAP III, p. 26, my emphasis). This was a top priority of further investigation, second only to testing the existing group for antibodies to Cry9C.

> **"The Panel felt that the medical community should be informed of the investigation into the allergenicity of Cry9C in corn products. In addition, monitoring reports from the medical community could supplement the cases currently under investigation and could provide additional support for proving or refuting the allergenicity of Cry9C."** (SAP III, p. 26)

Several suggestions for such monitoring came up during the SAP III meeting.

7.1 Alert the allergy community to the possibility of allergenic corn

> **"I think it would be useful at least to the allergy community to let them know … that there is even this possibility, because I think most of the time if somebody came and told me they were reacting to corn, I would be … very dubious about it. … I'm not saying that the Cry9C is an allergen necessarily, but if, in fact, it was, there would be no way for the allergy community to even validate any of that, unless they're aware that maybe they should contact an agency to say here's somebody complaining about a corn product…"** (Dr. Sampson, SAP Transcript, pp. 470-71)

Dr. Metcalfe concurs:

> **"…there are many organizations of physicians who deal with these kind of individuals [food allergy sufferers] and other groups of lay – lay groups that could get the word out that there was a website or something to go to to report these reactions."** (SAP Transcript, p. 470)

7.2 Test agricultural workers who have been exposed to StarLink corn for antibody response to Cry9C

> **"We were also told back in February [2000] that there were a number of workers that had handled this StarLink corn, had fairly heavy exposure. I don't know whether it's possible to get blood samples from them, but if they are identifiable,**

> **that was one of the suggestions back at that time that could be used."** (SAP Transcript, pp. 393-94)

As noted above, it appears that neither the government agencies nor Aventis followed up on this SAP I recommendation (SAP I, p. 9). A study of people with high-level occupational exposure to Cry9C corn could still be carried out. Such a study would provide valuable information on possible sensitization to Cry9C through the inhalant and dermal routes, two possibilities mentioned by both SAP I and III.

7.3 Additional testing

Given the special susceptibility of children to allergens, it would make sense to alert pediatricians to the possibility of allergenic corn (see Section 8). As mentioned above, animals that have been fed StarLink could also be tested for IgE antibody response to Cry9C:

> **"…we know animals have been fed this food for a while. We were told that it's very safe, it's not found in the meat, it's not found in the milk. It would not be difficult at all to look at the antibody response of these animals ingesting this food to know whether or not it has any immunogenic potential when it is ingested."** (SAP Transcript, Dr. Sampson, p. 393)

8. Infants and Children at Special Risk; Special Measures Needed

8.1 Novel proteins in genetically engineered foods raise special concerns with regard to children

Many studies indicate that children are 3-4 times as likely to suffer from allergies as adults. Therefore, exposure to novel food proteins such as Cry9C in genetically engineered foods is of special concern in the case of children:

> "Data from several studies estimate that 6-8% of children and 2-2.5% of adults have an immunologic-mediated reaction to foods… This suggests that **children are the population at most risk for the introduction of novel food proteins.**" (SAP III, p. 12)

8.2 Infants are still more susceptible

This is particularly true of children in the first two years of life. Speaking of this age group, Dr. Sampson states:

> **"…the younger child is definitely at higher risk for sensitization and likely…requires smaller amounts of a protein to cause a problem."** (SAP Transcript, p. 447)

Dr. Sampson notes that 95% of a group of 5000 peanut-allergic patients were sensitized under the age of 2, "and so most of these allergies are initiated in the first few years of life" (Ibid, 447-48). Dr. Rothenberg concurs, noting the special problem of exposure to reactive elements of novel proteins (new antigens):

> "The largest and highest concern is in the first two years of life, where there appears to be a higher incidence of allergic problems, **especially to new antigens that are encountered in the diet**…" (SAP Transcript, p. 437)

8.3 Infants and young children are also more sensitive

Not only are infants ***more prone*** to developing allergies to novel proteins, they are also ***more sensitive***, responding to lower levels and fewer exposures than adults:

> "…allergic responses can also be triggered by trace proteins following sporadic exposure, especially in young children." (SAP III, p. 16)

> **"…children exposed to Cry9C may be more sensitive than adults**." (SAP III, p. 14)

Breast-feeding infants, and even fetuses, may become sensitized to trace levels of food allergens:

> "… food allergy can be encountered in exclusively breast fed infants. Presumably, breast fed infants are exposed to trace levels of food antigens derived from the maternal diet or have been exposed *in utero*." (SAP III, p. 16)

According to Panel member Dr. Ricki Helm, a single exposure might be sufficient to induce an allergy:

> "It could take one instance of exposure to that food product; it may take several. We just have no knowledge at this point on levels of sensitization in subgroups or total predisposed individuals for IgE-mediated disease." (SAP Transcript, p. 446).

8.4 Infants often eat a disproportionate amount of corn

Not only are infants more likely to develop allergies, and at lower allergen levels and with fewer exposures than adults, they often consume more corn products (as a proportion of total diet) than adults. According to Dr. Sampson: **"…corn is something that's often put in formulas to thicken … so again, this is a group that may be at a little higher risk"** (SAP Transcript, p. 448). How much Cry9C are infants being exposed to? According to Dr. Rothenberg, we simply don't know:

> "I haven't seen definitive information about assessing what exactly is the level [of Cry9C], particularly in the first year of life, in baby foods that are containing corn products…" (SAP Transcript, p. 439)

Thus, it is not surprising that the Panel judged that: "Study of infant diets is therefore the highest priority." (SAP III, p. 14)

8.5 Highly food-allergic children – the most susceptible subgroup – also have the highest exposure

> "I think the other thing that concerns me as an individual who treats a lot of highly food-allergic children is that corn is the food that we use as our major food source. You know,

> people talk about small exposure. **In some of the children we deal with, they're basically ingesting an amino acid formula plus corn in a variety of ways, pasta, corn chips, corn flakes, grits, whatever. So, these children who are at highest risk for developing allergy are really getting way over the levels that people are predicting for corn, and if this is a potential allergen, that would be of great concern to me."** (Sampson, p. 395)

The full Panel concurs, citing the inadequacy of the EPA's Cry9C exposure estimates, which are based on USDA data on corn consumption for various subgroups:

> "However, the data base may not capture the small number of infants having severe allergenicity. For this group, much of the diet prescribed involves corn. Thus, these children could have an unusually high consumption rate. Also, there is a need to estimate Cry9C protein dietary exposure over a longer period of time." (SAP III, p. 23)

Thus, it is no surprise that Dr. Rothenberg recommends that exposure of infants to Cry9C be taken as the worst-case scenario: **"…infants are at the highest risk, so we need to really address this issue, and that will probably provide the worst case scenario for what exactly is the risk for sensitization."** (SAP Transcript, p. 438)

8.6 Food-allergic infants probably exposed to higher levels of Cry9C than estimated by EPA

As Dr. Sampson noted above (Section 8.5), food-allergic children often eat a disproportionate amount of corn, precisely because it is rarely allergenic and hence considered safer than other foods. Food-allergic infants are often put on special corn-based formulas in which potentially allergenic proteins have supposedly been broken down and rendered harmless (i.e. hydrolyzed). One example is Mead Johnson's Enfamil Nutramigen, a hypoallergenic infant formula that is sold in two forms: concentrated liquid and powder. Nutramigen powder contains 54% corn: 47% corn syrup solids and 7% modified corn starch (See Appendix V: Nutramigen Fact Sheet).

EPA considers the concentration of Cry9C in corn starch and corn syrup to be so low as to result in negligible exposure to the population, including infants (EPA White Paper, Tables 4 & 5, pp. 13-14). Yet there are a number of flaws in this analysis.

1) The Agency underestimated the amount of Cry9C in cornstarch by at least a factor of two by basing its calculation on the percentage of total protein in corn (8%) instead of soluble protein (4%) (EPA White Paper, pp. 10 & 13; Bucchini & Goldburg 2001).

2) The EPA has still failed to consider the exposure to Cry9C of food-allergic infants on diets extremely rich in corn, such as Nutramigen. As noted in the preceding section, this subpopulation has both extremely high potential exposure to Cry9C and extraordinary sensitivity to allergens, and is not captured in the USDA database used by the Agency. This oversight is surprising, given the EPA's admission that: "study of infant diets should be given high priority." (EPA Press Release 2000).

3) The Agency's data on daily exposure to Cry9C from cornstarch are presented in a misleading format in Tables 4 and 5 of the White Paper – in absolute microgram units rather than

micrograms/unit body weight. As a result, infants appear to have the lowest exposure to Cry9C, when in fact they are probably exposed to the highest dose on a body weight basis.

4) There is no attempt to account for infants' non-dietary exposure to Cry9C, despite the SAP's explicit recommendation to consider inhalational exposure in other contexts (e.g. agricultural workers & corn pollen/grain dust). This factor could be significant, since cornstarch is often used as a safe substitute for talc in cosmetic products for infants (Bucchini & Goldburg 2001), and could act synergistically with dietary exposure.

5) Two recent studies found unexpected corn proteins in Nutramigen, "presumably originating from the maize starch…" (Frisner et al, 2000). These proteins, identified as maize zeins, were purified and injected into rabbits, which developed antibodies to them. If immunologically active corn proteins can find their way into an infant formula specifically formulated to exclude them, this raises serious doubts about the efficacy of corn processing procedures in removing other proteins such as Cry9C.

Until the EPA conducts a thorough analysis of food-allergic infants' exposure to Cry9C, we will have come no closer to formulating the "worst case scenario for … the risk for sensitization" demanded by Dr. Rothenberg.

8.7 Special risks to infants and young children argue against granting any tolerance

Of course, children, infants and unborn children should ***never*** have been exposed to ***any*** Cry9C in corn. It is only thanks to the negligence of Aventis and its seed dealers, the naivete of the EPA in granting a split approval, and the failure of the FDA to monitor for Cry9C, that this massive experiment on America's children is currently underway.

Given the special risk factors associated with young children and infants – increased susceptibility to allergies, sensitivity to trace levels of food allergens, and greater dietary exposure to corn – approval of any sort of tolerance for Cry9C in the food supply is completely out of the question.

8.8 Special monitoring needed for young children/infants

Given the ongoing exposure of this high-risk group, special measures are called for to detect any possible cases of allergic sensitization/response to Cry9C among children and infants. The FDA is urged to: 1) Inform pediatricians and the allergy community of the possible presence of allergenic corn in infant formulae and other children's food; 2) Investigate reports of allergic reactions in food-allergic infants and children on corn-based diets to determine whether Cry9C is responsible; 3) Test a full range of corn-based infant formulae for the presence of Cry9C; 4) Conduct a prospective allergy study of food-allergic children on corn-based diets that may contain Cry9C.

Clearly, a testing regime which includes few if any young children/infants – the worst-case scenario for the risk of sensitization cited by Dr. Rothenberg – will never satisfactorily answer the question of whether Cry9C does or does not induce allergies.

9. Flaws in the FDA's Antibody-Testing Assay

9.1 A brief description of the ELISA

Another potentially serious problem with the FDA's allergy testing program involves the assay used to test for antibodies to Cry9C protein. These antibodies, known as immunoglobulin E (IgE), must recognize and bind to the allergen for an allergic response to occur. The relationship between an IgE antibody and its chosen allergen is like that of a lock to a key. Each antibody (normally) recognizes only one allergen. Even a minute change in the allergen's structure (key) can render it unrecognizable to its antibody (lock).

The assay used to test for IgE antibodies to Cry9C in the blood of those with suspected allergic reactions mimics this recognition & binding process outside the body. It is known as ELISA, or Enzyme-Linked ImmunoSorbent Assay. Basically, Cry9C protein is first attached to the 96 wells of a plastic plate. A test subject's blood serum is then added. Any antibodies to Cry9C in the serum will bind to it. Other antibodies will not bind. After washing to remove all unbound (and so non-Cry9C) antibodies, a solution of enzyme-linked "anti-IgE" antibodies is then added to the wells. Anti-IgE recognizes and binds only to IgE immunoglobulin in the wells. Since the only IgE present is bound to Cry9C, the quantity of anti-IgE that then becomes bound reflects the quantity of Cry9C antibodies in the wells, and by extension in the test serum. How is this measured? The enzymes linked to the anti-IgE antibodies catalyze a color reaction after the enzyme's substrate is added to the wells. The magnitude of the resulting color change (if any) reflects the quantity of substrate degraded, which in turn indicates precisely how many enzyme-linked anti-IgE antibodies (and so Cry9C antibodies) are present. The amount of color is measured by a special ELISA plate reader. This particular ELISA is known as a "double-sandwich" type because the Cry9C antibody is sandwiched between Cry9C protein below and enzyme-linked anti-IgE above.

Given the specificity of IgE antibody for the features of its chosen allergen, it is extremely important that the allergen attached the plastic plate be present in its natural form, unaltered in any way. This is where the difficulties begin.

9.2 Use of surrogate proteins for safety testing a controversial practice

The Cry9C used in the FDA's ELISA assay was produced in bacteria, not StarLink corn. This was accomplished by splicing the cry9c gene sequence into E. coli, which was used as a "bioreactor" to cheaply produce large quantities of a bacterial version of StarLink's suspect protein. The only reason given by the FDA for this choice is that "isolation of Cry9C from corn was not practical in the quantities needed to set up the ELISA method" (FDA Development 2001, p. 8). Yet even StarLink's original developer, Plant Genetic Systems (PGS), admits that "plant-derived material is probably best suited" for safety studies (Peferoen 1997a, p. 9).

This use of a surrogate protein for safety testing purposes is a common but often illegitimate practice in the biotechnology industry. It is supposed to be permitted only if the bacterial test protein is completely identical to the actual protein produced in the genetically engineered plant. According to an expert committee of the European Commission:

> "Extrapolating from the tested behavior of an isolated protein produced in a bacterium to predicting the behavior of the same protein when it is an integral part of the transgenic plant can be accepted only if the chemical identity (including conformational identity) of the two proteins has been demonstrated." (EC Scientific Steering Committee, p. 9).

The National Academy of Sciences, in its exhaustive review of Bt and other genetically engineered, pest-protected crops, takes a similar stance: "Tests should preferably be conducted with the protein as produced in the plant." If this is difficult due to the low levels of expression of the protein in question:

> "The EPA should provide clear, scientifically justifiable criteria for establishing biochemical and functional equivalency when registrants request permission to test non plant-expressed proteins in lieu of plant-expressed proteins." (NAS 2000, p. 65)

The EPA has failed to provide such criteria, despite the fact that surrogate proteins have been used in virtually all of the studies submitted for all of the Bt crops approved to date, including those for StarLink corn.

Use of the plant-produced protein is especially critical for allergy-testing purposes. According to allergy expert Dr. J. M. Wal:

> "The first critical point is the strict chemical identity of the foreign protein which is expressed in the genetically modified plant and of the test protein which is used for allergy assessment experiments.… slight structural changes may occur depending on the producing organism. These can affect the primary sequence of the protein due to point mutations on a few amino acid residues…"

As an example, he points to the different allergenic properties of two forms of a cow milk protein (β-lactoglobulin) that differ by only two amino acids (Wal, 1998, p. 418). As noted above (Section 2.2), the modification of a single amino acid in Cry9C to produce the lysine mutant form of the protein used in StarLink resulted in increased resistance to digestion by the protein enzyme trypsin.

9.3 Is plant-produced Cry9C equivalent to its bacterial surrogate?

Apparently, only one study has been done that specifically addresses this question (Peferoen 1997b). Plant Genetic Systems compared Cry9C protein extracted from corn with two surrogate proteins produced by bacteria genetically engineered with the cry9c gene sequence: 1) E. coli, and 2) a modified version of *Bacillus thuringiensis* stripped of its native protoxin cry9c sequence. The author discovered three differences:

1) The N-terminal amino acid sequences of the two bacterial proteins were identical, but differed from corn-derived Cry9C by 1-4 amino acids at the N-terminal

2) The molecular weights of the proteins differed substantially:

Corn-produced Cry9C:	73.8 kD	
Bt-produced surrogate:	70.5 kD	(3.3 kD or 4.5% lower than corn-produced Cry9C)
E. coli-produced surrogate:	67.9 kD	(5.9 kD or 8% lower than corn-produced Cry9C)

3) Corn-produced Cry9C was "weakly glycosylated," unlike either of the bacterial surrogate proteins. (Glycosylation refers to the attachment of carbohydrate groups to a protein).

E. coli bacteria do not have the capacity to glycosylate proteins, whereas plants do (Jenkins et al, 1996, p. 976). Thus, the higher molecular weight of corn Cry9C is probably due to the attachment of carbohydrate groups by the corn's cellular machinery.

According to Peferoen: "The nature of the glycosylation is under evaluation. The type of glycosylation will be determined using N- or O-glycosylation specific glycolases" (Peferoen 1997a, p. 15). That was in 1997. Aventis has yet to make this research available, despite the EPA's explicit request for more glycosylation data (personal communication, John Kough, EPA) and the lapse of four years.

As noted above, scientists have called for demonstration of "chemical identity" of plant and surrogate proteins as a precondition for use of the latter in safety testing. E. coli-produced Cry9C clearly does not meet this criterion. It also fails to meet all but one of the five criteria for test substance equivalence recommended by an EPA Scientific Advisory Panel examining mammalian toxicity of plant pesticides (SAP II, p. 14). SAP II explicitly recommends elucidating the full amino acid sequence of plant and bacterial test proteins to determine whether the two proteins are identical; "highly undesirable is the sequence analysis of 10-15 N and/or C-terminal amino acids," the method used by PGS. SAP II also demands that the two proteins exhibit "identical patterns of post-translational modification (i.e. glycosylation)" before the bacterial surrogate protein be accepted for testing purposes (Ibid, p. 14).

The only other indication of whether Cry9C is glycosylated comes from Dr. Metcalfe, who at the SAP hearing of Nov. 28th asked FDA's Dr. Kough whether a difference of 10,000 kD between the weights of Cry9C derived from data on "taco shell model residues" and "the Cry9 protein that's been tested" was evidence of glycosylation. Dr. Kough was unable to give a definitive response (SAP Transcript, p. 46).

9.4 Glycosylation and allergenicity

Glycosylation is considered to be a characteristic property of allergenic proteins. "The allergenic fraction of food is generally comprised of heat-stable, water-soluble glycoproteins…" (Sampson 1999). There have been many reports of IgE antibodies that bind specifically to certain carbohydrate groups of glycoproteins (as cited in SAP II, p. 23). The carbohydrate groups of glycoproteins of the α-amylase inhibitor family found in wheat and barley have been implicated in bakers' asthma, an allergic disease involving production of IgE antibodies (Garcia-Casado 1996). Similar carbohydrate groups appear to be involved in the IgE response of atopic individuals to glycoproteins present in tomatoes (Zeleny et al 1999, p. 208) and potatoes (Sepp[redacted] 2001, p. 52). In addition, a major soybean allergen (Gly m Bd 30K) has been found to be an N-linked glycoprotein (Bando et al 1996; see Section 9.5)

Glycosylation might also have an ***indirect*** effect on the allergenicity of a glycoprotein: "The presence of the carbohydrate groups on mature proteins affects several biochemical properties that increase the likelihood of allergenicity. A general character of glycoproteins is their highly increased solubility, proteolytic and thermal stability as compared to the unglycosylated counterpart." (SAP II, p. 23)

Glycosylation can affect the folding of the protein into its 3-dimensional configuration (Rayon et al, 1998). Differences in folding between native glycosylated and bacterial unglycosylated versions of the "same" protein "may in turn lead to a lack of immunological identity between the sensitizing allergen (native, glycosylated protein) and the [bacterial] recombinant allergen…" (Sepp[redacted], 2001, p. 16). Sepp[redacted] (p. 52) also reports immunoblotting results from Alibhai et al (2000) showing that IgE binding to a deglycosylated

recombinant potato allergen (rSol t 1) was reduced in comparison to the native patatin glycoprotein (Sol t 1). In still another example, **"…the enzyme amylase expressed in tobacco reacted with specific antiserum, while the same amylase expressed in a bacterial system did not."** (Consumer & Biotechnology Foundation, section 5.2.1).

These reports raise serious doubts about the FDA's ELISA assay, as they clearly indicate that glycosylated proteins and their unglycosylated bacterial surrogates can have greatly different immunological reactivity. Even the FDA admits this critical flaw in its ELISA:

> "Another potential problem was related to the use of recombinant Cry9C expressed in (i.e. derived from) the bacterium *Escherichia coli*. Recombinant proteins from this source are not glycosylated (i.e. they do not have carbohydrate molecules attached). The same protein expressed in the corn plant may be glycosylated. In the case of some allergens, the molecular structures recognized by IgE antibodies (epitopes) involve these carbohydrate molecules. **Thus, it is possible that epitopes present on Cry9C in corn may not be present in the *E. coli*-derived protein.**" (FDA Development 2000, p. 8)

In other words, antibodies formed in a presumed allergic reaction to StarLink Cry9C might not recognize the *E. coli* Cry9C used to test for allergies, resulting in false negative results. Until the exact nature of Cry9C glycosylation is determined, the validity of the results from FDA's antibody assay must remain in doubt.

9.5 N-linked glycosylation

In the glycosylation process, carbohydrate groups attach to specific sites on the amino acid backbone of the protein molecule. In the type of glycosylation most closely linked to allergenicity (N-linked glycosylation), the carbohydrate group attaches to the Nitrogen atom of asparagine residues occurring in the three amino-acid sequence Asn-X-Ser/Thr, where Asn stands for asparagine, X signifies any amino acid, and the third position is occupied by either serine or threonine (Jenkins et al., 1996, pp. 975-6; Bardor et al., 1999, p. 376). Examination of the amino acid sequence of Cry9C (as deduced from its DNA sequence) reveals **nine** such amino acid triplets in the expressed portion of the protein (Lambert et al., 1996, Fig. 2, p. 83). If one excludes sequences with proline or aspartic acid in the second position, as suggested by one team (Haruko & Haruko 1999, p. 413), there are still five N-linked glycosylation sites (see Appendix VI). While this does not prove Cry9C glycosylation is N-linked (the type associated with allergenicity), it demonstrates that there are 5-9 sites where this could occur.

9.6 Another Cry protein has been shown to be glycosylated

Two studies have demonstrated that the purified endotoxin from *Bacillus thuringiensis subsp. israelensis* (Bti) is a glycoprotein (Pfannensteil et al 1987; Muthukumar & Nickerson 1987). According to Pfannenstiel et al., Bti crystal toxin contains 1.7% amino sugars, comprised of 70% glucosamine and 30% galactosamine. The presence of these sugars was confirmed by strong binding of fluorescent wheat germ agglutinin, a lectin which recognizes N-acetylglucosamine (GlcNAc) and GlcNAc oligomers. N-acetylglucosamine is an important component of a group of complex-type N-linked glycans (containing $\beta 1 \rightarrow$ 2 xylose and/or $\alpha 1 \rightarrow 3$ fucose groups), which have been associated with the allergenicity of α-amylase inhibitors of wheat and barley (Garcia-Casado 1996) as well as immunogenic responses to ragweed and many other pollens. Muthukumar & Nickerson likewise demonstrated that the N-acetylglucosamine-specific lectin, wheat germ agglutinin, binds to the glycoprotein toxin of Bti; this binding was greatly reduced by treatment with periodate, confirming that the amino sugars are critical for binding.

Analysis of the Bti toxin proteins reveals sites suitable for N-linked glycosylation. While sequence homology between Cry proteins is not necessarily high, this clear evidence for glycosylation of a protein in the same family as Cry9C once again demonstrates the need to fully characterize StarLink Cry9C.

9.7 Other possible post-translational modifications

There might also be other differences between corn-derived and bacterial surrogate Cry9C proteins that haven't been discovered yet. Until this protein is fully characterized, we will have to rely on guesswork. This is the approach taken by Marnix Peferoen in the test substance equivalence study mentioned above, in which he compared corn-derived Cry9C to bacterial versions produced in E. coli and Bt Cry minus strains.

9.7.1 Phosphorylation

Like glycosylation, protein phosphorylation is a post-translational modification that can have immunologic consequences. One study has demonstrated that phosphorylated seryl residues are involved in the allergenicity of caseins (milk proteins). Casein peptides containing the major sites of phosphorylation appear to be important immunoreactive regions within the molecule (Otani et al, 1991). Bernard et al (2000) reached similar results. In their study, dephosphorylation of several naturally phosphorylated casein variants altered the binding capacity of IgE from the sera of milk allergic patients as measured by ELISA. Differences in IgE binding were also observed between phosphorylated and dephosphorylated tryptic fragments of beta-casein.

Unfortunately, it seems that no one has attempted to determine whether Cry9C is phosphorylated. According to Peferoen, phosphorylation is often dependent on interaction with specific protein kinases. He did not analyze possible phosphorylation of Cry9C because of "the limited knowledge of these proteins in plants in general" and the lack of commercial sources for plant protein kinases (Peferoen 1997a, p. 16).

Yet there is at least one report in the literature of Bt protein phosphorylation (Watson & Mann, 1988). Watson & Mann detected at least 14 phosphopolypeptides by pulse labeling of Bt kurstaki HD-1 Dipel with [32P] orthophosphate. Several of the phosphopolypeptides co-purified with the endotoxin crystal; the phosphoamino acid residue of the most abundant phosphopolypeptide (Mr 25,000) was identified as phosphothreonine. Watson and Mann note

that phosphopolypeptides (probably this same Mr 25,000) are also present in several other subspecies of Bt.

9.7.2 N-acetylation

While Peferoen does not test for N-acetylation, he considers it "most likely" that the NH_2 group at the N-terminal residue is N-acetylated because a large fraction of the Cry9C protein is not sequencable by the Edman degradation method (Peferoen 1997a, p. 15).

9.8 Conclusion

EPA and FDA officials still express uncertainty about the nature of Cry9C glycosylation. Although the issue first arose in the 1997 study by PGS (Peferoen 1997a), there has been no resolution of this important question by Aventis or the government. Based on the available information, corn-produced Cry9C and its bacterial surrogates are different in at least two respects relevant to the allergenicity assessment of Cry9C: molecular weight and glycosylation. Based on its deduced amino acid sequence, Cry9C also possesses 5-9 sites for the N-linked glycosylation most associated with allergenicity. A related Bt protein from subspecies israelensis is glycosylated, and also possesses N-linked glycosylation sites. Other possible post-translational modifications of StarLink Cry9C have not been adequately investigated. Finally, Aventis has failed to even confirm the deduced primary structure of StarLink Cry9C by sequencing its amino acids, despite the fact that "slight structural changes may occur depending on the producing organism" (Wal 1998, p. 418).

If the people who were tested by the FDA are in fact allergic to the Cry9C in StarLink corn, their sera might not recognize the surrogate bacterial protein used in the FDA's ELISA due to the differences noted above, or yet undiscovered ones. This would then result in "false negatives," faulty results indicating no allergic reaction when in fact one had occurred.

Given the importance that will likely be accorded the FDA's allergy testing results in the decision on whether to approve a tolerance for Cry9C in the food supply, it is critical to ensure that these results are valid. The best course would be to repeat the allergy testing with Cry9C derived from StarLink, in line with the recommendations of the National Academy of Sciences and the allergy experts cited above. At the very least, the EPA and FDA should do whatever studies are required to fully characterize the two apparently different Cry9C proteins from StarLink and *E. coli.* Only if they are chemically identical, or meet the strictest standards for test substance equivalence, should the present FDA assay results be accepted. Granting any tolerance for Cry9C in the food supply should not even be considered until such research has been conducted.

10. Aventis' History of Misconduct and Non-Cooperation

Aventis has petitioned the EPA to grant a tolerance of 20 ppb for Cry9C residues in food products. This petition must not be granted. There are simply too many unanswered questions, too many sources of uncertainty, to be able to conclude that Cry9C residues do not pose an allergenic risk to the American public. These uncertainties have been discussed in detail above. They include:

1) Exposure estimates that are little better than speculation;
2) Aventis' failure to characterize the Cry9C protein produced in StarLink;
3) The FDA's flawed allergy assay, which makes use of an E. coli surrogate protein rather than Cry9C from StarLink;
4) The government's failure to expand the investigation as recommended by the StarLink Scientific Advisory Panel;
5) The failure to account for the extra susceptibility of infants and children to potential sensitization by Cry9C;
6) Failure to follow-up on collection of additional antigenicity data (from workers exposed occupationally to Cry9C, from animals fed Cry9C corn)

In addition to these issues, other more general considerations should be taken into account. First, Aventis has demonstrated a pattern of irresponsible conduct for which it is clearly unrepentant. This is evidenced by its current attempt to foist responsibility for StarLink contamination onto the EPA and fate.

> "The possible presence of Cry9C protein in food is not a result of any misuse. Rather, it is the unavoidable and unforeseeable consequence of the combination of the split registration granted by EPA, and other factors beyond the control of Aventis" (Aventis Petition, p. 62).

This outright denial of responsibility bodes ill for Aventis' future conduct with respect to genetically engineered crops. And it also contrasts sharply with the company's earlier admission that it bore final responsibility for ensuring proper use of StarLink: "Obviously, we failed in some way at that task" (Aventis spokesman Rick Roundtree, as quoted in The Des Moines Register, 10/25/01; see Appendix VII).

Aventis' earlier position is the correct one. Contamination occurred primarily because Aventis violated its stewardship agreement with the EPA by failing to ensure that farmers were informed of the restrictions imposed by the EPA on growing StarLink: the limitation to animal feed and industrial uses, and the 660-foot buffer strip to be planted around plots of StarLink. In fact, Aventis and/or its agents apparently even deliberately misled farmers by attaching fraudulent tags on StarLink seed bags which indicated that the corn was suitable for human food use: "You are licensed upon purchase of this product only to produce forage or grain for *food*, feed or *grain processing*" (my emphasis). Iowa Attorney General Tom Miller says that most StarLink growers who called his office were not aware of the restrictions. He also offers a common-sense explanation for this failure to inform farmers: "I just don't think if the restrictions were disclosed many farmers would have bought the grain" (Ibid). Thus, Aventis and its agents (e.g. Garst Seed Company) had a clear financial motive to conceal the restrictions from farmers and even deliberately mislead them – namely, to sell more seed. And this is apparently exactly what they did.

Other "corporate character" issues should also be considered. As detailed above (Section 4.6), the company disregarded the explicit recommendations of two StarLink Scientific Advisory Panels for collection of more antigenicity/allergenicity data. For instance, SAP I outlined a monitoring program that Aventis could have undertaken in collaboration with its primary StarLink agent, Garst Seed Company, to investigate possible allergic reactions in workers exposed to StarLink pollen and/or grain dust. The call to examine occupational exposure was

repeated by SAP III as its second-highest priority for more data, and included the suggestion that serum samples be collected from these same workers and tested for antibodies to Cry9C. The need for these measures was strengthened by the discovery of IgE antibody response in workers exposed to Bt spray preparations (Bernstein 1999). Despite these recommendations, the reams of data submitted by Aventis since the SAP III meeting include nothing on occupational exposure to Cry9C. If Aventis had made a good faith effort to help supply such data, the question of Cry9C's allergenicity might have been settled by now.

But Aventis has not acted in good faith, as further demonstrated by the fact that the Cry9C protein in StarLink was intentionally engineered to be more resistant to digestion – one of the key characteristic properties of food allergens. This cavalier disregard of allergy concerns was aggravated by Aventis' failure to characterize the Cry9C protein. The company has apparently sequenced only the 10-15 amino acid residues at the N-terminal, and failed to establish whether the protein is acetylated or phosphorylated (Section 9). Despite knowledge of Cry9C's glycosylation by no later than 1997, and a clear statement to the effect that further studies were underway to characterize it, ***Aventis has failed to release any more data on Cry9C glycosylation*** despite explicit requests to do so by the EPA.

Thus, Aventis sought approval of StarLink corn for food use with full knowledge that its Cry9C protein had been engineered with a trait more likely to make it allergenic, then failed to characterize the protein to determine whether or not it was in fact allergenic. Nearly all tests conducted by Aventis, its predecessor companies, and the government – including the FDA's allergy test – have utilized the E. coli-produced surrogate protein, not StarLink Cry9C, despite evidence that the latter is substantially different and more likely to be allergenic.

Finally, even the data that Aventis did submit are seriously flawed. For example, buried deep in its 170-page study on Cry9C protein levels in food products, one discovers a serious breach of standard operating procedure in the processing of StarLink corn that was made into dry-milled corn products that were tested for their Cry9C content. Instead of the standard 30 minute heat treatment, this batch of StarLink was heated for two hours – four times as long – surely resulting in a much greater degree of Cry9C degradation than standard food processing procedures. Aventis does not explain this lapse, nor why the processing was not repeated with a new batch of corn heated for the proper period of time.

Neither is any adequate explanation given for the two- to nine-fold differences between two similar assays used to measure levels of Cry9C in these same dry-milled food products.

11. Conclusion

While superficially impressive, the evidence to support the conclusion that Cry9C does not pose an allergenic risk to the public is undermined at every turn by inadequate or faulty data, unproven assumptions, badly flawed test protocols, and the petitioning company's refusal to supply the most needed information. This last factor would not be so critical if our regulatory agencies had the expertise, resources and/or willingness to fill at least those data gaps that can be filled. Unfortunately, they do not.

The StarLink debacle is a case study in the near total dependence of our regulatory agencies on the "regulated" biotech and food industries. If industry chooses to submit faulty, unpublishable studies, it does so without consequence. If it should respond to an agency request with deficient data, it does so without reprimand or follow-up (e.g. statistics on allergic reactions reported to food companies). If a company finds it disadvantageous to characterize its product, then its properties remain uncertain or unknown. If a corporation chooses to ignore scientifically

sound testing standards (e.g. by using surrogate protein without first establishing test substance equivalence), then faulty tests are conducted instead, and the results are considered legitimate. In the area of genetically engineered food regulation, the "competent" agencies rarely if ever (know how to) conduct independent research to verify or supplement industry findings.

Two examples will suffice to demonstrate this. First, the so-called "FDA" allergy test is primarily Aventis' creation due to the simple fact that the critical reagents were supplied by the company. There is no evidence that the FDA made any attempt to independently verify the composition or purity of the Aventis-supplied, E. coli-derived Cry9C protein or the antibodies raised to it in animals. "Conflict of interest" is apparently not a concept the agency is familiar with. And although the FDA acknowledges the inadequacy of E. coli Cry9C for allergy testing purposes, it made no effort to purify Cry9C from StarLink itself, and develop a proper assay based on the protein people were actually exposed to.

One possible reason for this lapse is the FDA's avowed "cheerleader" role in promoting biotechnology. Since a proper assay would more likely turn up an allergy "problem," perhaps FDA chose the easy course of reliance on Aventis to avoid making trouble for the industry it openly promotes. This would be in keeping with the agency's history of subservience to the biotech and food industries with respect to genetically engineered foods. As noted by Henry Miller, head of biotech at the FDA from 1979 to 1994 and a strong supporter of genetically engineered foods:

> ***"In this area [biotech foods regulation], the U.S. government agencies have done exactly what big agribusiness has asked them to do and told them to do."*** (as quoted in The New York Times, 1/25/01).

Alternately, perhaps the government simply did not have the resources or the expertise to isolate or characterize corn Cry9C.

This latter possibility is suggested by a second example of regulatory incompetence. When the testing commissioned by Friends of the Earth and Genetically Engineered Food Alert first revealed Cry9C contamination of food products on September 18, 2000, it took the FDA nearly a week even to request a sample for confirmation of the testing lab's results. We later learned that this delay was not due to oversight, but rather to the simple fact that ***after two years of StarLink cultivation on hundreds of thousands of acres across the country, the FDA still did not have the expertise to even test for this potentially allergenic protein***. The agency had to call in Aventis, the regulated company, to teach it how to test for the regulated protein, Cry9C (personal communication, Eric Flamm, FDA).

Until the government finds the will and the resources to begin seriously regulating genetically engineered foods, future StarLink fiascoes are almost assured. Tens of thousands of field trials of biotech plants have been conducted or are currently underway across the country (Caplan 2001), some involving crops that produce drugs, such as antithrombin, a blood thinner, or cholera toxin, an adjuvant used for vaccines. Many of these plant-produced drugs – such as a vaccine for transmissible gastroenteritis in pigs currently being field-tested – are designed to elicit immune system responses when administered orally. The single most popular crop for such experiments is wind-pollinated corn. The USDA has done little to regulate these pharmaceutical plant trials, despite the admonitory example of StarLink. There is no requirement even to mark field-trial plots, on-site government oversight is minimal to non-existent, most of the drug genes spliced into plants are kept secret as "confidential business information," and both neighboring farmers and the public-at-large are kept in the dark about

where the trials take place. The USDA dramatically weakened biotech crop field trial regulation some years ago with its streamlined "notification" procedure that dispenses with the former requirement for an environmental assessment.

The decision taken on Aventis' petition for a tolerance for Cry9C will send a strong signal to the biotech industry concerning the government's (lack of) seriousness about biotech crop regulation. Granting the petition will encourage the biotech industry in the belief that it can flaunt even the already lax regulations and get away with it. This is a frightening prospect when one considers the drug-producing corn and other crops already in the fields. It is still more disturbing in light of Aventis' denial of responsibility for StarLink contamination, as well as the biotech industry's avowed strategy of spreading genetic contamination throughout our agricultural lands as quickly and widely as possible so as to preempt labeling and other needed regulatory initiatives. According to biotech industry consultant Don Westfall:

> ***"The hope of the industry is that over time the market is so flooded that there's nothing you can do about it. You just sort of surrender."*** (as quoted in The Toronto Star, 1/9/01).

Granting this petition would not only needlessly endanger public health, particularly the health of infants and children, but it would also send an unmistakable signal of regulatory surrender to the biotech industry. Friends of the Earth and Genetically Engineered Food Alert strongly urge the EPA to deny Aventis' petition for a tolerance for Cry9C protein.

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Appendix I: Foods Subject to Recall Due to Cry9C Contamination

(Data drawn from 7 FDA Enforcement Reports: dated 10/4/00, 11/1/00, 11/15/00, 12/6/00, 1/24/01, 1/31/01 and 4/11/01; available on the FDA's website at www.fda.gov)

Recall Initiated	Product(s)	Manufacturer	Distribution	Quantity
9/22/00	Taco Bell Home Originals: Taco shells and taco dinners	Sabritas Mexicali (owned by PepsiCo); distributed by Kraft Foods	Nationwide.	635,991 cases
10/4/00	Cornmeal, corn flour, snack meal, flaking grits, polenta grits, pregel/cereal binder under various brand names: Cahokia Pride, Dixianna, ConAgra, USA Cornmeal, Sysco Classic, Alberto A-1	ConAgra Corn Processing	MO, TX, PA, UT, IA, TN, M	1,450,484 lbs.
10/13/00	Tortillas, taco shells, tostadas, chips. Supplied to supermarkets, restaurants and food service establishments under brand names such as Mission, Safeway, Albertson's, Best Buy, Campbell's, Food Lion, Kroger, Shaw's, Western Family, Guerrero's, Diane's and many others	Mission Foods of Irving, Texas. Mission is the largest supplier of corn products in the country, and makes products for supermarkets, which carry them under their own names	Nationwide, Canada, Korea	**Quantity undetermined** Supermarket: 200 products under 70 brand names Restaurant/food service: 97 products under 17 brand names
10/13/00	Maseca yellow corn flour; Masa Mixta All yellow corn flour manufactured since 1/1/00	Azteca Milling (sister company to Mission Foods)	Nationwide and international	**Quantity undetermined**
10/18/00	Bulk dry soup mixes: Creamy Chicken Noodle Soup & corn Chowder Soup	John B. Sanfilippo & Son, Elk Grove Village, IL	Pennsylvania, Ohio, Kansas, Iowa	883 pounds
11/2/00	Lynn Wilson's Stone Ground Corn Tortillas: 8, 11 & 32 ounces sizes	Wilson Foods Company, Salt Lake City, Utah	Utah, Idaho, Montana, Washington state	1,672 cases
11/6/00	SYSCO Cheese Enchiladas	Fernando's Food Corporation of Compton, CA	California	1,415 cases
11/6/00	Yellow Corn Flour masa to make taco shells, tortillas, tortilla chips, corn chips and taquitos	Minsa Corporation of Muleshoe, Texas	Nationwide	56,045 x 50 lb. bags = 280,225 lbs.
11/22/00	Lauhoff Tiny Flakes, used for brewing beer	Bunge Lauhoff Grain Company of Crete, Nebraska and St. Louis, MO	Pennsylvania and Wisconsin	64,350 lbs
1/11/01	Enriched Scott's brand Pearl Plain Yellow Cornmeal	Scott's Auburn Mills, Inc. of Auburn, Kentucky	Illinois	**Quantity not cited** One lot of 50 lb. bags
3/14/01	Morningstar Farms and Loma Linda brand meat-free/veggie corn dogs; Morningstar Farms Party Pack (Chick Nuggets, Mini Corn Dogs, Buffalo Wings)	Alpete Meats, Muncie, IN Recalled by Kellogg Company of Battle Creek, MI	United States, Canada, Caribbean	441,206 cases
3/29/01	Carroll Shelby's Original Texas Brand Chili Kit	Reily Foods Company of New Orleans, LA	Nationwide	723,192 units (4 oz. bags)

Breakdown of Products Subject to Recall

The following totals exclude 3 recalls due to lack of data: Mission Foods (likely the biggest recall of all), Azteca Milling and Scott's Auburn Mills. More precise accounting of the available data is impossible due to failure to report number of items per case for all products.

Supermarket products: 1,080,284 cases
723,192 items

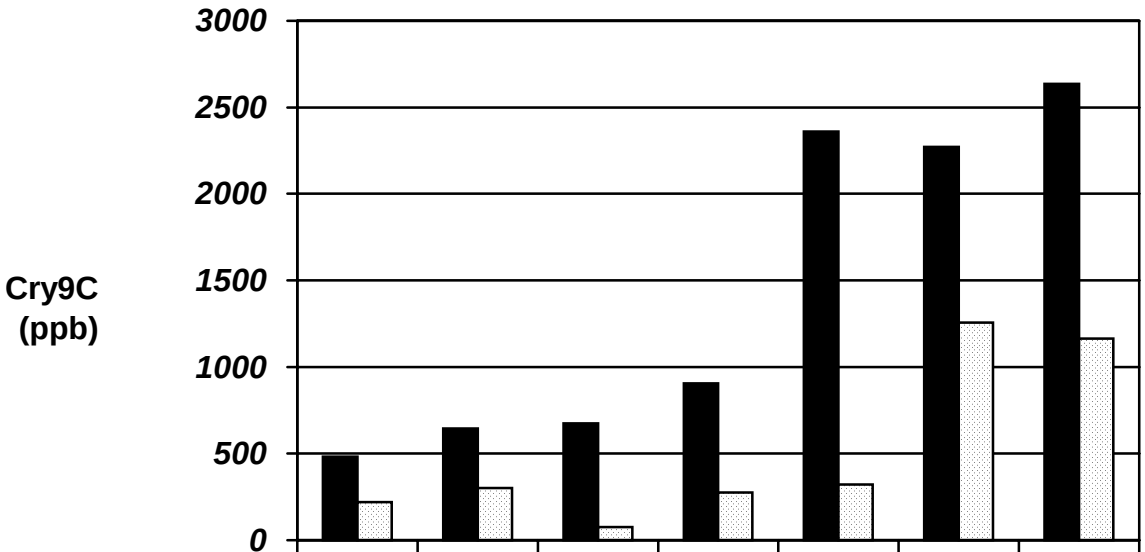
Bulk Foods: 1,795,942 lbs.

Appendix II: Interassay Comparison of ELISAs in the Measurement of Cry9C

In a study by Aventis, two ELISA assays were used to measure Cry9C protein levels in various corn fractions and products made from StarLink corn: a commercial assay from EnviroLogix and an in-house ELISA developed by Aventis (Aventis Detection 2001). Both assays employ polyclonal capture and detection antibodies raised against bacterially-expressed Cry9C. Below we present the discrepancies between these two assays for a range of dry-milled corn products. The paired bars for each food product represent the test results of the two assays ***for the same sample***. Each value (bar) represents the average of four measurements: 2 measurements on each of two 1 gram subsamples.

Cry9C Protein in Dry-Milled Corn Products Made From StarLink Interassay Comparison: EnviroLogix v. Aventis

(based on data from Aventis Detection, 2001, Table 9, p. 38)



	Polenta 1	Polenta 2	Muffins 1	Muffins 2	Corn bread 1	Corn bread 2	Hush Puppies
EnviroLogix	483	645	674	906	2,361	2,273	2,636
Aventis In-House	219	302	76	275	322	1,257	1,163

Table 1: Interassay Comparison of EnviroLogix and Aventis ELISAs for Dry-Milled Corn Products. Each column represents one sample. Each figure represents the average and standard deviation of 4 Cry9C protein measurements: 2 measurements on each of two 1 gram subsamples.

ELISA / Corn Product	Polenta 1	Polenta 2	Corn Muffins 1	Corn Muffins 2	Cornbread 1	Cornbread 2	Hush Puppies
EnviroLogix	483 ± 32.5	645 ± 93.7	674 ± 422	906 ± 475	2,361 ± 206	2,273 ± 194	2,636 ± 158
Aventis	219 ± 31.4	302 ± 41.2	76 ± 10.5	275 ± 15.4	322 ± 56.1	1,257 ± 162	1,163 ± 55.7
% Increase*	121%	114%	787%	229%	633%	81%	127%

* Percentage denotes amount by which EnviroLogix results exceeds Aventis ELISA results.

Appendix III: Suspected Allergic Reactions to StarLink with FDA Report Dates from November 30, 2000 to January 8, 2001

(from FDA Consumer Complaint/Injury Report Forms)

No.	Complaint date/status	Report date	Suspected product(s)	Product ID/sample?	Description
527	11/17; in progress-pending evaluation	12/19; no follow-up	Mission tortilla round chips	Code 03YFT09 Lot 01NOV0500B51L 1; also UPC, PAC	Man. Migraine, nausea, slight fever > eating product. No meds while eating product. Takes glaucoma medication. No known food/drug allergies. Contacted firm, which picked up sample for analysis.
623	11/22; in progress-pending evaluation	12/19; no follow-up	Salad shells at Taco Bell restaurant	None	Cramps and diarrhea for several days after eating salad shells at Taco Bell
739	11/30; pending at branch	11/30; no follow- up	Tortillas, brand not given	None	Man. Employer phoned CDC, thought employee experiencing allergic reaction to tortillas. Saw doctor, treated with Benadryl and perhaps more.
746	11/30; in progress-pending evaluation	12/01; no follow-up	Kraft Taco Bell taco shells	Code 07BGT02 Lot 2XSB7	Woman. Arch began hurting, red & swollen. Dr. gave steroid shot and prescript. Dr. thought allergy to some food, possibly taco shells. **Woman contacted attorney, who had taco shell analyzed, shown to contain StarLink**. Occurred 8/26/00
765	12/01; in progress-pending evaluation	12/04; no follow-up	Tyson Mexican Original Enchilada style corn tortillas (yellow corn)	Code 03YGY09 Lot 506; consumer brought in open bag to FDA	Man. Ongoing rash; at times difficulty breathing. Rash 1 ½ h > eating; diff. breath. 3 h >.. Happened twice, both times eaten w/ ham & cheese. Treated at hospitals (where he works as X-ray tech) and by private dermatologist. Received Ranitidine, Loratadine, Allegra. Allergy to dog/cat/horse hair. No meds for allergy. Rash still on legs.
772	11/28; follow-up requested; occurred 8/1/00, in hospital 8/1 to 8/6/00	12/4	Frito Lay Tostito corn chips	Code 07BFT02 Lot 5673119113N; 5 oz. remaining	Woman. Ate product w/ refried bean dip before retiring. Wheezing 10 pm; gasping for breath 4 am; taken to ER, diagnosed w/ congestive heart failure/acute respiratory distress. She disagrees, thinks it was allergic reaction. No food allergies or meds before incident. FDA cites "severe respiratory distress syndrome/bronchoconstriction or bronchospasm." FDA says "medical records do not need to be collected at this time."
833	12/6	12/19; closed	Frito-Lay Tostito corn chips	None	Man. GI distress, contact dermatitis. Very unlikely case.
859	12/7	12/19; closed	Kellogg's corn flakes	Code 05AFT01 Lot KLB-008	Complainant's wife reports he experienced stomach cramps, nausea and chills > eating corn flakes. Contacted Kellogg's. FDA contacted Giant where product purchased, no other complaints received on product/lot.
863	12/7	12/19; closed w/o investigation	Mission Yellow corn tortilla chips, 1 lb.	Code 03YGT; ate 20%, discarded rest.	Hives and itching after consuming product. Insufficient information, unable to evaluate. No lot codes, cannot locate manufacturing location.
901	11/30	12/19; closed	Corn	None	Change in body weight. GI distress. Insufficient information; unable to evaluate.
909	12/11	12/19; pending at branch	Burrito dinner at Taco Bell	None	Ate burrito dinner and "immediately" developed hives. Previously eaten same thing many times w/o problems. Rash & hives on and off since then for ½ year. Saw dermatologist, received antihistamines, helped but not cured. Lip swelling on occasion > Taco Bell meal; once > Mission tortillas. In Nov. 2000, advised by dermatologist to suspend antihist's prior to RAST; but 1 day > discontinuing antihist's, tongue, lips and throat swelled, taken to ER, treated w/ prednisone, etc. RAST then done, negative. Back on antihist > ER visit. EPA referred him to Karl Klontz; e-mail from Klontz to Sandi Hanson not available. Man gave FDA permission to have personal identifiers sent to CDC.
1305	1/4/01	1/8/01; closed	Purina One dry cat food; Real Chicken and Rice Special Formula for adult cats	Code 72AF-02 Lot U 1031 L4	Cat w/ diarrhea since Sept 2000 while on product. Diarrhea undiagnosed by vet. After taking cat off corn-based cat food 11/29, no more diarrhea. Purina confirms corn is in this product. Complaint sent to CVM FYI. Cat taken to Shake Veterinary Hospital (address given)

Appendix IV: Suspected Allergic Reactions to StarLink Corn Reported by Consumers to Food Companies

The following summary covers allergy reports from the following sources: Reports contained in EPA docket number OOP-00688 (Nov. 28th SAP); materials obtained from the FDA by Joe Mendelsohn, attorney at the Center for Food Safety, through a Freedom of Information Act request.

Food Industry Balks at Supplying Information Needed for StarLink Allergy Investigation

On October 30, 2000, the FDA, EPA and USDA sent a joint letter to four food industry trade groups requesting "information concerning possible allergic reactions by consumers that could be related to the presence of StarLink in processed food." "The purpose of this letter is to enlist your immediate assistance in requesting that *your member companies submit promptly to the FDA* any such information in their possession that is reasonably related to StarLink. The information specified above need not be sent if the allegation concerned a product in which the only processed corn ingredient was one of the following which do not retain protein: (i) corn syrup, (ii) corn starch, (iii) ethanol, and (iv) corn oil that is not cold processed." These four food industry trade groups were the National Food Processors Association (NFPA), Grocery Manufacturers of America (GMA), Food Marketing Institute (FMI) and Snack Food Association (SFA). (Joint agency letter, 10/30/00, my emphasis)

To our knowledge, no company except Mission Foods (see below) responded directly to the FDA, as requested in the joint agency letter. Other companies sent information to NFPA or GMA instead. (We have not discovered responses from FMI or SFA or their member companies in the materials cited above.) NFPA and GMA then submitted to the FDA heavily redacted summaries of whatever information they had received from member companies, removing company names and aggregating the allergic reaction data. These summary reports, which are discussed in more detail below, are wholly inadequate for several reasons: 1) The agencies requested that the *companies themselves* send data, not the trade groups; 2) The agencies requested *any* information on possible allergic reactions that could be related to StarLink, not redacted summaries of said information; 3) According to the FDA, and as demonstrated below, the GMA and NFPA submissions and other industry reports lack critical data – even such basic information as name, contact information and description of symptoms – which prevented follow-up and evaluation of the allergic complaints as had been done for reports submitted directly to the FDA (FDA Evaluation 2001, pp. 3-4).

While the summary information supplied by the NFPA and GMA was useless for case evaluation purposes, the original allergic reaction reports made by consumers to food companies might very well provide sufficient detail for follow-up and evaluation. All that would be needed to permit follow-up is the name and telephone number of the complainant. Unfortunately, the FDA has not sought to obtain these original allergic reaction reports from the food companies. Yet this is precisely the information that the agencies originally requested, and it is what should have been supplied. Liability concerns on the part of the food industry must not be used as an excuse to obstruct investigation into a public health threat. The FDA and EPA are urged to do whatever is necessary to obtain these original reports in order to supplement the handful of allergic reaction complaints made to the FDA that have been investigated.

Analysis of Allergy Report Summaries Supplied by NFPA and GMA

In evaluating the following data, please note that Sept. 18th, 2000 is the date of the first disclosure of Cry9C contamination of food products (Kraft Taco Bell taco shells). The significance of the increase in the number of allergic complaints following 9/18/00 was explained by Dr. Hugh Sampson at the StarLink Scientific Advisory Panel hearing on 11/28/00.

> **"It was suggested that until the first reports of the Cry9C came out in the press, nobody really reported adverse reactions to corn. That to me is not surprising. I think that the majority of people don't in any way suspect corn as being a major allergen and would have no reason to suspect that any kind of adverse reaction associated with a meal in which they ingested corn would provide a problem. So, I don't think the fact that nothing came up before that should be considered as a reason to believe that these reactions are not, in fact, real."** (SAP Transcript, p. 461)

Also, please recall that the companies were informed that they need not supply data concerning allegations of allergic reactions to products containing only corn ingredients less likely to contain appreciable amounts of Cry9C: corn syrup, cornstarch, ethanol or corn oil. Thus, the complaint data likely involve corn products with relatively higher levels of protein (and hence Cry9C).

Submission (11/27/00) to FDA from the National Food Processors Association

* Contains data pooled from 11 unnamed food processing companies over the period from 1998 to November 17, 2000.

* There were 31,580 allergy/health-related consumer contacts with these 11 companies in the 2-month period from 9/18-11/17/00. This compares to an average *annual* figure of 14,112 contacts for the period 1998-9/17/00. In other words, **the frequency of consumer allergy/health contacts rose 1,239% (over 13 times) in this 2-month period.**

* Of these 31,580 contacts, ***210 involved allergy complaints in which yellow-corn containing products were specifically mentioned*****.** This compares to an average *annual* figure of 14 in the preceding period. **In other words, *yellow-corn related allergy complaints were 90 times more frequent in this 2-month period*.**

* **Of the 210, nearly half (45%) sought medical attention:**
  + 53 spoke with a company-retained physician, who "found no confirmed cases of allergic reaction." Thus, a telephone diagnosis by a doctor on the company payroll failed to *confirm* an allergic reaction. This is clearly not an adequate investigation of these allergy complaints, especially given the conflict of interest situation of the company-retained physician
  + ***74 (35%) "sought medical treatment with a physician"***
  + ***20 (9.5%) "sought medical attention in an emergency room"***
  + 72 had no contact with a physician
  + "1 consumer had a sudden onset of rheumatoid arthritis. Her doctor believes it may be from GE ingredients."

* Of the 210 corn-related allergy reports, NFPA says that consumers reported eating other foods/ingredients in combination with the corn product *in every single case*. In the preceding 2 ¾-year period, 4 of the 39 complaints (10%) involved the yellow corn product alone. Based

on history, then, one would expect 10%, or 21 of the 210 corn-related allergy reports to relate to corn products alone. The absence of a single complaint to corn products alone in such a large group is therefore highly unexpected.

Submission to the FDA from the Grocery Manufacturers of America (11/8/00)

* GMA received information covering 1998, 1999 and 2000 from 9 major food companies.

* GMA passed on the information to its legal counsel, which then passed it on to an unnamed firm or individual [name blacked out] for analysis and aggregation.

* Data and/or brief statements are given for each unnamed company. No data are submitted for three of the nine companies. Where available, the data are highly statistical in nature; in some cases, even the number of corn-related allergic complaints actually received is missing.

* **Data from at least two companies directly contradict the claim in the submission that there was no increase in reports (i.e. complaints & inquiries) concerning potential allergic reactions to foods that contain corn over the last several years.**

  + Company Three breaks down data for 1998, 1999 and for two periods in 2000 (before and after 9/18). Allergy figures include those for products that may not contain corn. The company claims no consumer reports that could reasonably be related to StarLink corn, but the data belie this:
    * **The frequency of allergy complaints in the period 9/18 - 10/30/00 rose 226% from the average for the period from 1998 to 9/17/00.**

  + Company Seven reported only calls that pertained to allergic reactions to yellow-corn containing products. Results were reported as ratio of complaints to "MM lbs. product" (the actual number of complaints is not cited).
    * **Complaint frequency per pound of product in the period 9/17 to 10/28/00 rose 277% over the period from 1998 to 9/16/00.**

  + Company Nine: The full response is: "[W]e have not had a single consumer call claiming to have an allergy to Cry9 protein." This response leaves unclear whether the company received any allergic complaints mentioning "StarLink" or corn products that might have contained it.

  The other responses do not seem to indicate an increase in allergy reports after 9/18/00, but the data/statements are of very poor quality and make any definitive conclusions impossible.

  + Company One simply reports that >0.05% of 950,000 "annual consumer contacts" related to alleged allergies, and that the number has been consistent over the past 3 years.

  + Company Two reports 2 complaints in 2000 for "corn based cake." "[N]either appears to be related to corn; Complaint rate in 2000 is 1 per 1.6 million units." Data for ice cream novelty products and seafood products do not show an increased frequency of allergy complaints in the year 2000.

  + Company Four's data consists of "total number of health effects" and "# of corn-related" broken down by year; the strange thing here is the large numbers of "health effects"

reported: 1998: 3114, 1999: 1455, 1/1/00-9/17/00: 594, 9/18-10/30/00: 95. In other words, large drops in complaint frequency from 1998 to 1999 to the period from 1/1/00 to 9/17/00. Then, a slight increase in complaint frequency for 9/18/00-10/30/00. Only 2 cases are reported as corn-related over the entire period.

+ Company Five: The only response given is: "We have received no complaints of adverse reactions which could reasonably be related to StarLink corn."

+ Company Six: 47 illness complaints over the entire period from 1998 to 2000 year-to-date, with only 4 of these allergic reaction complaints. The company reports annual sales of about 100 million units.

+ Company Eight: The company reports "no complaints of allergic reaction attributable to products in which yellow corn is an ingredient from June 1, 2000 through October 31, 2000. During the same period we have no record of any consumer inquiries mentioning StarLink."

Letter from Mission Foods (12/22/00) to Joseph Baca, FDA in response to the FDA/EPA request for consumer complaints that may be related to StarLink

* Mission Foods did not respond until after the meeting of the StarLink Scientific Advisory Panel on November 28, 2000

* By that date, Mission had received 3,400 phone calls responding to the StarLink press reports

* "Since press reports regarding this issue first emerged in October [sic], **Mission Foods has received approximately 20 calls from consumers inquiring about a physical effect possibly caused by consumption of a yellow corn product.**"

* Physical effects "range from common, flu-like symptoms to rashes or headaches." The company downplays their significance, citing other possible causes.

* "The company continues to investigate these reports, but does not believe that any of its yellow corn products caused any of the reported physical effects, or that any reports are reasonably related to the consumption of StarLink corn."

* Mission promises to contact each consumer who reported a possible physical effect caused by consumption of a Mission Foods yellow corn product and tell them they may report information by calling the FDA (888) INFO-FDA. Some did not leave contact information.

Appendix V: Composition of Nutramigen Infant Formula

(http://www.meadjohnson.com/products/hcp-infant/pnutram.html)

Enfamil Nutramigen

HYPOALLERGENIC PROTEIN HYDROLYSATE FORMULA

Hypoallergenic formula supplying protein in hydrolyzed form, for infants and children sensitive to intact proteins of milk and other foods.

COMPOSITION

Ingredients: Concentrated Liquid (diluted with equal parts water) and Ready-To-Use: Water (87%), corn syrup solids (6%), vegetable oil (palm olein, soy, coconut, and high oleic sunflower oils) (3%), casein hydrolysate (2%), modified corn starch (2%), and less than 1% vitamin A palmitate, vitamin D3, vitamin E acetate, vitamin K1, thiamin hydrochloride, riboflavin, vitamin B6 hydrochloride, vitamin B12, niacinamide, folic acid, calcium pantothenate, biotin, ascorbic acid, choline chloride, inositol, calcium carbonate, calcium phosphate, magnesium oxide, ferrous sulfate, zinc sulfate, manganese sulfate, cupric sulfate, sodium iodide, sodium citrate, potassium chloride, potassium citrate, sodium selenite, acetylated monoglycerides, carrageenan, citric acid, L-cystine, L-tyrosine, L-tryptophan, taurine, L-carnitine.

Ingredients: Powder: **Corn syrup solids (47%)**, vegetable oil (palm olein, soy, coconut, and high oleic sunflower oils) (24%), casein hydrolysate (17%), **modified corn starch (7%)**, and less than 2% vitamin A palmitate, vitamin D3, vitamin E acetate, vitamin K1, thiamin hydrochloride, riboflavin, vitamin B6 hydrochloride, vitamin B12, niacinamide, folic acid, calcium pantothenate, biotin, ascorbic acid, choline chloride, inositol, calcium citrate, calcium hydroxide, calcium phosphate, magnesium oxide, ferrous sulfate, zinc sulfate, manganese sulfate, cupric sulfate, sodium iodide, sodium citrate, potassium citrate, potassium chloride, sodium selenite, acetylated monoglycerides, L-cystine, L-tyrosine, L-tryptophan, taurine, L-carnitine.

NUTRIENTS (Normal Dilution)	Per 100 Calories (5 fl oz)
Protein, g	2.8
Fat, g	5
Carbohydrate, g	11
Water, g	134
Linoleic acid, mg	820
Vitamins	
Vitamin A, IU	300
Vitamin D, IU	60
Vitamin E, IU	2
Vitamin K, µg	8
Thiamin (Vitamin B1), µg	80
Riboflavin (Vitamin B2), µg	90
Vitamin B6, µg	60
Vitamin B12, µg	0.3
Niacin, µg	1000
Folic acid (Folacin), µg	16

Pantothenic acid, µg	500
Biotin, µg	3
Vitamin C (Ascorbic acid), mg	12
Choline, mg	12
Inositol, mg	17
Minerals	
Calcium, mg	94
Phosphorus, mg	63
Magnesium, mg	11
Iron, mg	1.8
Zinc, mg	1
Manganese, µg	25
Copper, µg	75
Iodine, µg	15
Selenium, µg	2.8
Sodium, mg	47
Potassium, mg	110
Chloride, mg	86

INDICATIONS

Feeding of infants and children sensitive to intact proteins of milk and of other foods
Feeding of infants with severe or multiple food allergies
Feeding of infants with colic, persistent diarrhea, or other gastrointestinal disturbances due to milk protein allergy
Maintenance of nutrition during test or elimination diets
Tube feedings for patients with food allergies
Feeding of infants with galactosemia

RATIONALE AND SPECIAL CHARACTERISTICS

Nutramigen is a hypoallergenic, lactose-free, sucrose-free formula well-utilized by the infant allergic to protein. The protein of Nutramigen is supplied as hydrolyzed casein specially processed to be essentially non-antigenic. The other ingredients are also selected for hypoallergenicity. During the time Nutramigen is fed for the nutritional management of infants, Nutramigen can:

Effectively eliminate the symptoms of protein allergy

Allow the sensitive G.I. tract to recover

Eliminate the need for “trial and error” search for an acceptable alternative formula

Establish and maintain a healthy nutritional state during recovery

Plus, Nutramigen is well-accepted and well-tolerated by infants. Whole protein may not be digested efficiently when the gastrointestinal mucosa is temporarily compromised due to protein allergy or infection (as evidenced by prolonged or severe diarrhea), or the integrity of the mucosa is compromised as a result of surgery. Development of allergy may result should a portion of the protein be absorbed intact.

Appendix VI: Cry9C Protein Sites Suitable for N-Linked Glycosylation

(from Lambert et al 1996, Figure 2, p. 83)

FIG. 2. Nucleotide and deduced amino acid sequence of the *cry9Ca1* operon. Underlined DNA and amino acid sequences indicate relevant features explained in the text. Further details are given in Results and Discussion.

See Section 9.5 for explanation of N-linked glycosylation:

Circled: Commonly accepted sequence for N-linked glycosylation

Boxed: More restrictive sequence proposed by Haruko & Haruko (1999).

Appendix VII: Growers of StarLink Not Warned

Growers of biotech corn say they weren't warned
StarLink tags appear to indicate it's suitable for human food products

By WILLIAM RYBERG, Des Moines Register Business Writer, 10/25/2000

Butler County farmer Jim Norton says he received no special warnings last spring when he purchased seed for StarLink, the corn that has prompted the recall of taco shells and other corn-based food products.

He said the fine print on a tag attached to the bag appears to say that corn grown from the genetically engineered seed can be used for food. Norton planted 115 acres of the corn. Now, he has more than 20,000 bushels of it standing in the field while he tries to figure out what to do with it. StarLink is the corn that has not been approved for human consumption, which is why food products that contain it have been recalled.

StarLink's manufacturer, North Carolina-based Aventis CropScience, has said that farmers were supposed to have been warned when they purchased the seed that they needed to keep the corn separate from other corn that might end up as human food.

The reason for the segregation is that StarLink contains a protein that can cause allergic reactions in humans. Government officials have said there is no evidence of health problems associated with the corn, but they are concerned because it has shown up in food-processing plants, where it's not supposed to be.

Norton, who farms near Clarksville, north of Waterloo, said he was never told to segregate StarLink from other corn, and it now appears that many other farmers were not told either. An estimated 9 million bushels of StarLink corn are unaccounted for and are believed to have entered a storage and transportation system through which they could end up in food-processing plants. No one is sure how many farmers are in Norton's situation.

A spokesman said Iowa Attorney General Tom Miller's office had received about a dozen calls from StarLink growers. "Most tell us they were not told about the restriction," said Miller aide Bob Brammer. The attorney general's office is trying to help farmers and grain elevators deal with the problem.

The situation has caused confusion for farmers who grew StarLink corn and uncertainty for grain-elevator operators who may have mixed it with other corn because they did not know that StarLink needed to segregated.

Miller said the complicated restrictions associated with StarLink raise a common-sense question: Why would farmers buy the seed if they knew there were so many conditions

attached to growing the crop? "I just don't think if the restrictions were disclosed many farmers would have bought the grain," Miller said.

Norton said he thought StarLink could be grown and sold like any other corn. **A tag on the seed bags seemed to say so, Norton said. It says: "You are licensed upon purchase of this product only to produce forage or grain for food, feed or grain processing."**

A spokesman for Garst Seed Co. of Slater, the company that sold most of the StarLink seed in Iowa, said seed dealers were advised of the restrictions, and one tag on seed bags told farmers to check a grower's guide about restrictions.

Jim Erickson, the dealer who sold the seed to Norton, said he didn't recall receiving any information about restrictions. He said the seed bags bore nothing that he considered to be a warning label. Erickson is manager of the Fredericksburg Farmers Co-op.

Rick Roundtree, a spokesman for StarLink developer Aventis, said he had been told that a majority of some 3,200 growers nationwide signed agreements promising to grow the crop according to the restrictions.

Aventis estimates that StarLink was planted on about 135,000 acres in Iowa this year. The acreage makes up 40 percent of the cropland planted to StarLink nationally. The Iowa acreage represents only 1 percent of the state's corn acreage.

Norton said the StarLink he purchased was a good buy, $56 a bag, compared with common prices of about $120. The discount was part of a package deal that included buying herbicide designed to be used with StarLink.

Jeff Lacina, a Garst spokesman, said Garst had sent information on restrictions to seed dealers over the past two years. More than a dozen mailings have gone out to sales representatives, and information has been provided at sales meetings, Lacina said. A tag on seed sacks advised farmers to check a grower's guide for restrictions and gave a telephone number to obtain a guide if the farmer didn't have one, Lacina said.

Norton said he first learned of the restrictions when letters arrived a few weeks ago, explaining that Aventis was trying to isolate StarLink corn to assure that it didn't get into human food. Aventis spokesman Roundtree said seed companies that sold StarLink were responsible for telling growers about restrictions.

Aventis, however, bears final responsibility for seeing that the product is used as it should be, Roundtree said. "Obviously, we failed in some way at that task," he said.

Neil Hamilton, director of the agricultural law center at Drake University in Des Moines, said StarLink raises a variety of legal questions about liability.

"This is kind of one of those classic situations where lawyers are going to be hauled in to sort out what it means," Hamilton said.

Appendix VIII: FDA Confirms StarLink DNA in Latest Allergy Report

JUN 21 2001

Dr. Keith Finger
13519 U.S. Highway 1
Sebastian, FL 32958

RE: Consumer Complaint Sample Number: 3228
Product: Kash n Karry White Corn Tortilla Chips

Dear Dr. Finger:

FDA received your complaint alleging adverse events linked to food that may have been contaminated with StarLink™ corn. This letter is in follow-up to previous letters regarding the sample. We followed up on your complaint, collected a food sample, and analyzed it. We tested your sample for the presence of DNA of the gene coding for the pesticidal protein of StarLink™ corn. The gene was found. We also tested your sample for the presence of the pesticidal protein itself. The protein was not found.

If you have any further questions or concerns regarding this sample please call or write to:

Mr. Mark Hackman
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition, HFS-606
200 C St., S.W.
Washington, D.C. 20204
(202) 205-8211

Sincerely,

Frederick D. Teddy, D.V.M., M.S.
Acting Branch Chief
Import Programs Branch
Division of Enforcement and Programs
Office of Field Programs