

**Final Comments for Submission to  
the Environmental Protection Agency  
Docket No. OOP-00678B**

**Concerning the Revised Risks and Benefits  
Sections for *Bacillus thuringiensis* Plant-  
Pesticides**

by Bill Freese  
on behalf of Friends of the Earth  
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**NOTE:**

This submission supercedes comments submitted via e-mail on Sept. 10<sup>th</sup> with respect to the Bt corn products up for reregistration. It also contains full citations for all sources referenced, unlike the prior submission.

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# Key Findings

## Health Assessment of Bt Crops

- \* The EPA has failed to establish and/or apply standards for the health assessment of the genetically engineered Bt crops being considered for registration: three varieties of Bt corn, one each of cotton and potatoes (Sections 3 & 12)
- \* While the EPA is supposed to evaluate three parameters of allergenicity – digestive stability, resistance to heat and structural similarity to known allergens or toxins – the Agency has either failed to demand that companies submit corresponding studies, despite the lapse of 5 years since the original registrations, or the data submitted are of poor quality (Section 3, Table 2)
  - + **Amino acid sequence homology:** The EPA has collected virtually no data on potential similarities between the structure of Bt proteins and the structures of known allergens and toxins, even though all experts agree that this simple test should always be carried out on all genetically engineered foods, and even the EPA considers it to be “required” data (Section 4)
  - + **Digestive stability:** Two studies submitted to the EPA demonstrate that the Bt corn protein, Cry1Ab, exhibits digestive stability similar to that of StarLink corn’s Cry9C protein. This was one of the key findings that led the EPA to reject StarLink for human consumption (Section 6)
  - + **Heat stability:** Cry1Ab also exhibits stability to heat comparable to that of StarLink Cry9C. The EPA has accepted substandard tests for other Bt crops that fail to assess the parameter of interest – the degree to which the Bt protein is degraded by heat (Section 5)

## Deficient Product Characterization

- \* Many of the studies submitted by companies were conducted on Bt proteins from different crop lines, representing distinct transformation events, than the one registered, leading to the dubious practice of “data bridging.” (Section 9)
- \* Much of the safety testing was performed on truncated versions of bacterial surrogate proteins rather than the full-length, plant-produced Bt proteins people are actually exposed to. Studies supposedly demonstrating the equivalence of bacterial surrogate proteins to their plant-produced counterparts for the purpose of safety testing do not meet the standards set by EPA’s scientific advisors, and so should not be accepted (Section 10)
- \* The EPA relies unduly on data generated with Bt microbial pesticides, which differ in significant ways from the Bt crop-produced proteins (Sections 8 & 11)

- \* Monsanto's Yieldgard corn is the result of a transformation gone awry. While Monsanto intended to insert the full-length cry1Ab gene, apparently only a fragment was incorporated, for reasons unknown. Monsanto has had difficulty even detecting the 92 kD protein supposedly produced by this gene fragment. Yieldgard also lacks the foreign genes for the herbicide-resistance trait that were supposed to be inserted and were "apparently lost during the development of the MON 810 line." (Sections 1 and 8.2)
- \* The Agency has no data confirming the removal of the ampicillin resistance gene from the DNA used to transform Syngenta's Bt 11 corn (Section 1)

### **Procedural Violations**

- \* The EPA has largely ignored the recommendations of its scientific advisors (SAP Bt Plant-Pesticides, SAP Mammalian Toxicity), contrary to the Agency's claim that it has considered them (Sections 1 & 10)
- \* The EPA's Biopesticides Registration Action Document shows no evidence that the EPA has considered "the most current health and ecological data" or "all available scientific information on Bt products," the supposed purpose of this re-assessment
- \* "Required" data that should have been collected and carefully evaluated *before these crops were originally registered* are still missing/deficient despite the lapse of five years, and the EPA appears poised to compound this error by re-registering the crops, perhaps without time limit, in the absence of these "required" data (Section 3)

### **Data Apparently Not Considered**

- \* A 1999 study by Bernstein et al partly sponsored by the EPA suggesting that the Bt proteins Cry1Ab and Cry1Ac can elicit antibody (IgE) responses consistent with allergic reactions in farm-workers has apparently not been considered, despite the explicit recommendation by SAP Bt Plant-Pesticides that the Agency make use of serological agents developed in the course of this study to conduct "clinical assessment of exposed individuals" (Section 11)
- \* A series of 4 studies on mice dated 1999 and 2000 by Vazquez et al showing that Cry1Ac is a potent immunogen, increases antibody responses to other antigens, and binds to surface proteins in the mouse small intestine have also apparently been ignored. One of these studies was recommended by SAP Bt Plant-Pesticides for consideration by the EPA (Section 11)
- \* The Agency has had evidence since the 1994 that Bt protoxins differ immunologically from the truncated proteins used for testing purposes, yet apparently has not followed up on this important finding (Section 8)
- \* Evidence that the toxic portion of Cry1A proteins can have a different 3-D conformation depending on whether it is part of the protoxin or in its free state, and that DNA structurally associated with the protoxin is released during the proteolysis process that generates the toxic fragment from the protoxin has also gone unconsidered (Section 8).

## 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 (SAP StarLink II, p. 11; Sampson 1999), or about 8 million Americans children (based on US Census data). 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.

The Environmental Protection Agency is presently considering whether or not to re-register five genetically engineered Bt crops – three varieties of Bt corn and one each of cotton and potatoes – which produce insecticidal proteins derived from a soil bacterium known as *Bacillus thuringiensis*. Concern about the potential allergenicity of one variety of Bt corn, StarLink, led the EPA to deny approval of this crop for human food use. After the food supply nevertheless became contaminated with StarLink, a panel of experts that included some of the nation’s leading allergists advised the EPA that it was impossible to establish a threshold below which the StarLink protein, Cry9C, could be considered safe for human consumption. It was for this reason that the EPA refused to approve a petition by StarLink’s developer, Aventis CropScience, to allow even an extremely low level of Cry9C residues in the food supply.

In examining studies submitted to the EPA and recommendations by the Agency’s scientific advisors, Friends of the Earth has found evidence that the bacterial-derived insecticidal proteins produced by other Bt crop varieties either possess properties similar to those of Cry9C, or have not been assessed for the same. These allergenic properties include digestive stability, resistance to heat, and structural similarity to known allergens (or toxins).

In particular, studies conducted by Aventis CropScience and Dr. Hubert Noteborn demonstrate that the Cry1Ab toxin produced by the Bt corn varieties registered to Monsanto and Syngenta exhibit digestive stability that is similar to StarLink’s Cry9C toxin. Noteborn’s study also shows that Cry1Ab and Cry9C have comparable resistance to breakdown by heat. The Agency has apparently not collected any data on potential similarities between the Bt proteins and known allergens or toxins, aside perhaps from limited data on Bt potatoes (see Table 2).

Additional concerns are raised by the poor quality of the studies conducted by the registrant companies. In many cases, these studies were conducted on Bt proteins from different crop lines than the one that was registered. In addition, the bulk of safety testing was performed on truncated versions of bacterial surrogate proteins rather than the full-length plant-produced Bt proteins that people are actually exposed to. This use of surrogates for testing purposes is a controversial practice, and is supposed to be permitted only if the bacterial and plant-produced

versions are shown to be equivalent. The so-called “test substance equivalence” studies submitted by registrants do not meet the standards recommended by EPA’s expert advisors. Failure to establish such equivalence in the case of the StarLink protein, Cry9C, was a major reason the EPA refused to permit even low levels of this protein in the food supply.

In addition, several of the Bt crops did not turn out as planned by the registrants, casting doubt on their ability to control the crude “gene gun” transformation (i.e. engineering) process. Monsanto’s Yieldgard corn, for example, apparently contains only a fragment of the gene that was supposed to be inserted, due to an unexplained failure in the transformation process. The company has had difficulty even detecting the protein actually produced by this gene fragment, and has conducted its testing instead on the portion of this protein that is resistant to trypsin. Yieldgard corn also lacks the foreign gene for the herbicide-resistance trait that was supposed to be inserted, again for reasons unknown.

Both the registrants and the EPA make invalid comparisons between Bt microbial pesticides (i.e. Bt sprays) and the plant-produced Bt proteins to support their contention that the latter are safe, despite the fact that there are a number of important differences between the native Bt proteins and the modified versions produced in crops.

Finally, the EPA has apparently failed to consider important evidence demonstrating that Cry1Ab elicits antibody (IgE) responses consistent with an allergic response in farm-workers, as well as other studies demonstrating that the closely related Cry1Ac induces a potent antibody response in mice, increases the antibody response to other antigens, and binds to surface proteins on the mouse small intestine.

The seriously deficient data submitted by registrants raise questions about the potential health impacts of their Bt crops. The EPA’s flaccid acceptance of poorly conducted corporate studies, and its apparent unwillingness to demand “required” data, casts doubt on the entire regulatory process, which appears to be unduly biased towards approval of Bt crops in the absence of critical data.

The unanswered questions with respect to the potential health impacts of Bt crops take on added significance in the context of an increasing incidence of food allergy, whose causes remain unknown. The EPA is urged to conduct a thorough and rigorous re-assessment of the Bt crops being considered for re-registration by demanding that missing data be supplied, requiring that deficient studies be repeated under proper conditions, and giving full consideration to the recommendations of its expert scientific advisors. Re-registration should not even be considered until complete and accurate data have been collected and carefully evaluated.

## **1. Introduction**

The EPA's stated objective in its reassessment of Bt crops is "to assure that the decisions on the renewal of these registrations are based on *the most current health and ecological data.*" The reassessment is supposed to take account of "all available scientific information on *Bt* products," giving particular consideration to recommendations by the EPA's Scientific Advisory Panels (e.g. SAP Bt Plant-Pesticides 2000, SAP Mammalian Toxicity 2000) as well as the National Academy of Sciences' in-depth review of Bt crops (NAS 2000) (EPA BRAD 2001, p. 11, my emphasis).

A thorough-going reassessment is badly needed for several reasons. First, genetically engineered Bt crops are still a relative newcomer on the agricultural scene, having been approved for commercial planting barely more than half a decade ago. Since that time, there has been a great deal of research on these crops which should be considered before a decision is made on whether to allow their continued cultivation. Second, the science of genetic engineering is still primitive. The process of transgene insertion is crude and haphazard. As a result, even plant developers often lack complete data on their transgenic products. Pleiotropic (i.e. unexpected) effects are more common than once imagined, and often remain undiscovered for years, by which time the crop may have come to be cultivated on tens of millions of acres.

For example, since their introduction in 1994, evidence has accumulated that Roundup Ready (RR) soybeans, by far the most widely planted genetically engineered crop, have lower phytoestrogen levels (Lappe et al 1999); depressed root development, nodulation and nitrogen fixation; lower levels of aromatic amino acids; and lower average yields than their conventional counterparts (Benbrook 2001). In addition, the original promise of reduced herbicide use has proven false; in fact, cultivation of Roundup Ready soybeans is associated with application of greater amounts of herbicide, on average, than their conventional counterparts (Ibid). These hard *facts* – lower yields and increased herbicide use – contrast sharply with vague biotech industry *promises* that future GE crops will somehow "feed the world" and reduce agricultural chemical use. Finally, fragments of DNA undetected at the time of original registration were recently discovered in Monsanto's product, five years after the crop was first commercialized. Two of these fragments, 72 and 250 base pairs in length, appear to be partial copies of the CP4 EPSPS gene which lends RR soybeans their herbicide resistant trait (Monsanto RR Soybeans 2000). More recently, a Belgian team reported that a 534 base pair segment of DNA flanking the previously undetected 250 bp fragment does not match soybean genomic DNA, as expected, and suggested that it could be scrambled plant DNA or DNA from an unknown source (Windels et al 2001).

Scientists are only now beginning to unravel the molecular basis of the pleiotropic effects mentioned above. In order to do so, thorough and accurate product characterization data are essential. For this reason, one could argue that no genetically engineered crop should be in the fields or on the market until it has been completely and accurately characterized at the molecular level. At the very least, it seems hard to argue with the notion that regulatory authorities should demand the best information possible with the most modern techniques that are available. Some of the improved techniques employed by Monsanto and the Belgian team in obtaining more

accurate characterization of RR soybeans (e.g. genome walking, cosmid library construction and northern blot analysis) (Monsanto RR Soybeans 2000, p. 4) have apparently not been applied to the Bt products being considered for reregistration.

For instance, the Agency is set to re-register Syngenta's Bt 11 corn despite lack of data confirming removal of the ampicillin resistance gene from the transforming DNA (EPA BRAD 2001, p. IIA5). In the case of Monsanto's Bt corn, the EPA seems satisfied with the conclusion that: "These genes which confer glyphosate tolerance were apparently lost during the development of the MON 810 line..." (Ibid, p. IIA7). In addition, the Agency appears to be untroubled by the failed transformation event by which only a portion of the intended full-length cry1Ab gene sequence was actually incorporated into the genome of MON 810 corn, despite Monsanto's apparent difficulty in detecting the protein encoded by this unintended fragment (Ibid, p. IIA7; see Section 9.2).

The very prevalence of genetically engineered Bt crops is still another reason for a strict and thorough reassessment. Even if impacts on health and the environment prove to be minor, it must not be forgotten that we are dealing with crops planted on tens of millions of acres, to which large numbers of people are exposed. To take a concrete example: If StarLink's Cry9C protein is shown to be even a rare allergen, affecting just a tiny percentage of the population, the likelihood that tens of millions of people have consumed tainted corn products (Friends of the Earth 2001, pp. 14-15) suggests that a large number of people may have been affected. And if StarLink, which was never planted on more than 0.4% of total U.S. corn acreage, can spread so widely in the food system, it is reasonable to assume that the other Bt corn varieties, which constitute roughly 20% of U.S. corn production, have been consumed by nearly every American for several years now.

## **2. EPA's Criteria for its Human Health Assessment**

In assessing the potential of Bt crops to impact human health, the EPA has set out a short list of criteria that it considers relevant: digestive behavior, structure vis-à-vis the structures of known allergens and toxins, stability to heat and toxicity (EPA BRAD 2001, p. IIB1-2). These criteria are generally evaluated through unpublished performed by the registrant or a firm it has hired for the purpose.

While the presence of any of these traits – digestive stability, structural similarity to a known allergen or toxin, heat stability or toxicity in laboratory animals – serves as a red flag that the pertinent Bt protein could cause adverse human health effects, it is important to understand that the absence of one or several does not provide reasonable scientific certainty that the protein in question is safe. With respect to allergy, the simple tests employed for the first three criteria yield only suggestive evidence, and do not provide an adequate scientific basis for approving a crop – especially one grown and/or consumed on such a wide scale as Bt corn and cotton. For a higher degree of certainty, more extensive analysis is required – for instance, serum screening and tests with animal models (FAO/WHO 2001, pp. 11-13). An increasing number of authorities recommend post-marketing surveillance for adverse effects after market introduction as an additional safeguard (Wal 1998 & 2001; SAP StarLink I, p. 11; SAP StarLink II, p. 11;

Consumer & Biotechnology Foundation 1999, section 5.2; EC Scientific Steering Committee 2000, p. 11; FAO-WHO 2001, p. 9).

### **3. EPA Fails to Collect “Required” Data**

As long as the EPA is considering only the three inadequate, merely suggestive criteria cited above to identify potential health and allergenic concerns, the Agency should at the very least require that registrants conduct the pertinent tests rigorously, and submit complete data on the same, so as to provide the best chance of identifying potentially allergenic crops within the context of its seriously deficient regulatory framework.

Surprisingly, this has not been done. As shown by the table below, which is reproduced from the EPA’s Human Health Assessment BRAD (p. IIB3), *the Agency has failed to collect data it explicitly sets forth as “required for the Bt plant-pesticides to provide a reasonable certainty that no harm will result from the aggregate exposure to these proteins.”* (EPA BRAD, p. IIB1, my emphasis).

**Table B1 Confirmatory Studies Needed to Complete Product Database**

Common Name and Cry Protein	OPP Chemical Code	Study Type
Bt11, Cry1Ab Bt Corn	006444	Amino Acid Sequence Homology
MON810, Cry1Ab Bt Corn	006430	Amino Acid Sequence Homology/Processing and/or Heat Stability
Cry1Ac Bt Cotton	006445	Amino Acid Sequence Homology
Cry3A Potato	006432	Additional Amino Acid Sequence Homology / [Heat Stability]*

\* Though heat stability is not listed in Table B1 as lacking for Cry 3A potato, the EPA states on the preceding page that: “No heat stability studies were available for Cry3A.” (p. IIB2)

The EPA’s approach and language here are incomprehensible. The data listed above as lacking are explicitly stated to be “required” for a reasonable demonstration of safety. Thus, one would naturally expect such test results to have been submitted and carefully evaluated prior to the original registrations of these crops. Clearly, this did not take place. Still worse, *the very same data have not been submitted in the 5 years since the original registrations were issued.* Now, the EPA is poised to re-register these crops – perhaps without time limit – without having first collected or assessed the most basic data it explicitly “requires” concerning potential health impacts – much less consider the “most current” data or “all available information on Bt products” – the supposed purpose of this reassessment (EPA BRAD 2001, p. I1).

A clue to the Agency’s thinking is provided by the illogical table heading – “Confirmatory studies needed to complete product database.” The first question must be this: How can studies that the Agency “requires” to first determine whether or not a product is safe be in any sense “confirmatory”? What is being “confirmed” here? Is it perhaps the Agency’s pre-judgement, or prejudice, in favor of these crops in the absence of crucial data? In the concluding section, I will further explore the EPA’s apparent bias with respect to Bt crops.

Here, I will first examine the Bt crops up for reregistration to determine how well their respective Bt proteins meet the EPA's first three criteria (the ones relevant to potential allergenicity), making use of studies submitted by the registrants as well as other scientific information and recommendations from scientists and expert scientific bodies. Then, I will address other problematic aspects of the EPA's product characterization and health assessment, especially with respect to allergenicity.

#### **4. Amino Acid Homology to Known Allergens**

A novel, genetically engineered protein which is similar in structure (homologous) to a known allergen is generally thought to have a greater likelihood of being allergenic itself. This has led to the practice of comparing the (deduced) amino acid sequence of novel proteins with those of known allergens, a procedure which has been incorporated in practically every protocol for the allergenicity testing of genetically engineered proteins (e.g. Metcalfe et al 1996, ILSI 1996, Wal 1998, SAP Mammalian Toxicity 2000, FAO/WHO 2001). The National Academy of Sciences (2000) also recommends use of this predictive tool (Section 3.4.1).

***Metcalfe et al (1996, p. S167) recommend that all novel, genetically engineered gene products be subjected to this procedure, and tested for stability to digestion and processing as well*** (see Sections 5 and 6 below). In their scheme, any sequence of eight contiguous amino acids in the test protein that matches a corresponding sequence of a known allergen is cause for concern. This approach has been widely used since it was adopted in guidelines proposed by the International Life Sciences Institute in 1996 (so-called ILSI decision-tree). Appendix 1 contains several representative decision-tree approaches for testing genetically engineered proteins.

Since that time, accumulating knowledge has suggested various refinements and alterations to the ILSI decision-tree approach:

- 1) Allowance for substitution of chemically similar amino acids in the 8-amino acid sequence (SAP Mammalian Toxicity 2000, p. 21; Taylor 2001, p. 768);
- 2) 35% overall amino acid homology to a known allergen as an additional criterion (FAO-WHO 2001, p. 10);
- 3) Identity of 6 or 4 contiguous amino acids rather than 8 (Wal 1998, p. 418; Consumer and Biotechnology Foundation 1999, Section 5.1; SAP Mammalian Toxicity 2000, pp. 22-3; FAO-WHO 2001, p. 10; Becker 2001, p. 4);
- 4) Analysis of local alignments (regions with a high degree of similarity) rather than the entire protein when evolutionarily unrelated proteins are being compared (Gendel 1998);
- 5) Development of databases and methods to test for conformational or discontinuous epitopes (defined by 3-D structure rather than simple amino acid sequence) (SAP Mammalian Toxicity 2000, pp. 23-4; EC Scientific Steering Committee 2000, p. 9). While probably not as significant as continuous epitopes for food allergies, conformational epitopes have been discovered in several food allergens (Taylor 2001, p. 768), for instance the peanut allergen Ara h 1 (Shin et al 1998), and so must not be ignored.

The utility of the amino acid homology parameter is limited by several factors, most importantly the fact that the amino acid sequences of most allergens remain unknown. At present, full-length amino acid sequences are known for only 198 major allergens, among them just 30 food allergens (SAP Mammalian Toxicity 2000, p. 21). Thus, while a match raises concern and argues for further testing, failure to find a match to a known allergen is far from ruling out a test protein's potential for allergenicity (Becker 2001, p. 4).

Despite these limitations, the EPA has properly followed the recommendations of expert bodies and adopted amino acid homology comparisons to known allergens and toxins as one of four criteria in its human health assessment of Bt proteins (EPA BRAD, pp. IIB1-2). Unfortunately, however, the Agency does not specify any particular protocol for such testing. Presumably, it relies on the widely adopted ILSI decision-tree. Yet as noted above, many refinements and alterations have been suggested to improve this particular approach. The Agency does not appear to have evaluated these recommendations, as there is no discussion of them in its Human Health Assessment.

The point is moot, however, since *the EPA has not collected any data on potential sequence similarities between the Bt proteins of the four crops listed above and known allergens or toxins*, aside perhaps from limited data on Bt potatoes (Ibid, p. IIB3). The Agency's only explanation for this lapse is that registrants chose not to submit the pertinent studies.

“Amino acid homology comparisons for Cry1Ab, Cry1Ac and Cry3A against the database of known allergenic and toxic proteins were not submitted.” (EPA BRAD Human Health, p. IIB2)

It is unclear why the EPA did not *require* submission of such studies before it even considered approving these Bt crops in the first place. It is still less acceptable that it is not requiring these data now for the reassessment process. In these cases, not only has the Agency failed to consider “the most current health ... data,” much less “all available scientific information on Bt products,” it has not collected or evaluated any data whatsoever.

The EPA's failure to demand such studies is still more puzzling in light of the different treatment accorded Cry1F corn, for which the Agency apparently did receive and evaluate a study examining potential amino acid homology to known allergens and toxins (EPA BRAD Cry1F Corn, pp. 10, 15). While this study did not turn up any matches, it was based on a search for homologous sequences 8 amino acids in length. There is no evidence that the EPA has even considered the objections and suggested refinements to this technique cited above. Thus, in the case of Cry1F corn, the Agency has failed to meet its objective of considering the recommendations of its expert advisors.

## **5. Stability to Heat**

The stability of a protein to heat is considered by many to be a characteristic property of food allergens. “The allergenic fraction of food is generally comprised of heat-stable, water-soluble glycoproteins...” (Sampson 1999). Taylor & Hefle (2001, p. 768) also assume that food

allergens are stable to heat. The EPA concurs, citing stability to heat as a characteristic of allergenic proteins (EPA Cry1Ab [MON 810] Fact Sheet 2000).

The Agency has formally adopted heat stability as a second criterion of potential allergenicity (EPA BRAD 2001, p. IIB2), but has apparently provided no guidance on how Bt proteins should be tested for this property, much less a detailed test protocol. As with amino acid homology to known allergens and toxins, the Agency has also failed to demand data from registrants. Of the five Bt proteins being considered, apparently only one (Cry1F) was subjected to a heat stability test (EPA BRAD Cry1F Corn, p. 9). Two others – the Cry1Ab in Novartis corn and the hybrid Cry1Ac/Ab in Monsanto cotton – were “demonstrated to be inactive in processed corn or cottonseed meal.” This apparently means that the Agency accepts processing studies as a substitute for heat stability testing. The EPA has received no heat stability studies for either Cry3A (potatoes) or Monsanto’s Cry1Ab (MON 810 corn) (Ibid, pp. IIB2, 3).

Because processing methods can vary widely, the EPA should not accept processing studies in lieu of controlled heat stability tests carried out in accordance with a rigorous and repeatable test protocol. Dr. Ricki Helm recently emphasized the need for standardization in this area:

“The definition of heat stability should be standardized using the following criteria.

- 1) Heat treatment of the novel protein, native and recombinant, should be for 5 minutes at 90° C.
- 2) Assessment of stability by a combination of molecular sieving using HPLC and standardized SDS-PAGE analysis (both native and denaturing gels).” (Helm also outlines an SDS-PAGE protocol) (Helm 2001, pp. 9-10)

Contrary to Helm’s recommendation, the processing studies mentioned above apparently employed “inactivity” as the sole criterion to evaluate degradation – presumably as measured by bioassay in target insect species. This is also the only method used to assess “degradation” in the Cry1F protein heat stability study. The problem with using insecticidal activity as the sole parameter of heat stability is the implicit assumption that the insecticidal mode of action is relevant to potential allergenicity, and that loss of insecticidal activity somehow correlates with loss of allergenic potential. This assumption does not appear to be warranted, since it is the size of the breakdown fragments, not (loss of) insecticidal activity, which is of interest for allergenic potential. Loss of insecticidal activity could involve nothing more than (partial) denaturation, with little or no breakdown of the protein’s primary amino acid structure. This probably explains why Helm recommends techniques (HPLC, SDS-PAGE) to directly measure the size of fragments (if any) resulting from the heating process, and does not mention bioassays at all.

Thus, the finding that: “Cry1F protein was labile to heat [after 30 minutes] at and above 75° C” as measured by “growth inhibition of neonate tobacco budworm larvae” following “application of treated Cry1F to the surface of an insect diet” (EPA BRAD Cry1F Corn, pp. 9, 10) provides little or no useful information on the degree of fragmentation (if any) that is of interest for Cry1F’s allergenic potential.

Though the registrants either failed to submit heat stability studies at all or used improper assessment methods, there is one study available that measures fragmentation of Cry1Ab, the protein expressed in Novartis Bt11 and MON 810 corn, upon heating. This study was actually

conducted with the main objective of measuring the heat stability of StarLink's Cry9C protein, but it employed Cry1Ab for purposes of comparison. It was submitted to the EPA in 1998 by AgrEvo.

Dr. Hubert Noteborn, the author of this study and an expert in the field of Cry proteins, concluded that:

*“Studying the Cry1Ab5 protein a relatively significant thermostability was observed which was comparable to that of the Lys mutant Cry9C protein (Figure 6).”* (Noteborn 1998, p. 22, my emphasis)

Concerning Cry9C, Noteborn stated that:

“The protein was stable for 120 min independent of whether Cry9C was assayed as a purified protein or as a component of TM [tomato matrix]...” (Ibid, p. 22)

In this study, Noteborn assessed heat degradation by means of SDS-PAGE stained with silver, in line with Helm's recommendation cited above. He exposed 50 µl samples of Cry9C, Cry1Ab5 and other reference proteins diluted to a concentration of 0.7-0.8 mg/ml in water, buffer and/or tomato matrices (pH = 7.5) to a temperature of 90° C for 5 minutes to 210 minutes, and then ran these samples on an SDS-PAGE gel to measure the degree of degradation. Unfortunately, Dr. Noteborn does not elaborate on the heat stability of Cry1Ab5 beyond the quote cited above, perhaps because the study was focused on Cry9C, and the reproduction of the gel (Noteborn's Figure 6) available to me is of poor quality and does not permit more detailed analysis.

The heat stability of Cry9C was one of the characteristics that raised concern about StarLink corn's allergenic potential. The “comparable” heat stability of Cry1Ab5 should raise similar concerns about MON 810 and Bt 11, two of the Bt corn products up for reregistration. The heat stability study conducted on Cry1F should be repeated using SDS-PAGE or HPLC rather than insect bioassay to measure degradation. Similar tests should be demanded for Cry3A potatoes and BollGard cotton's hybrid Cry1Ab/Ac. Because the latter protein is so similar to heat-resistant Cry1Ab, one might expect it to be heat-stable as well.

## **6. Digestive Stability**

### ***6.1 Digestibility as a characteristic of allergenicity***

Resistance to digestion is generally regarded as a key characteristic of many important food allergens. Conceptually, this is based on the fact that “...larger non-digestible proteins or protein fragments have a much more prolonged capacity to reach the immune system the longer they are in evidence in the intestinal tract.” (SAP Bt Plant-Pesticides, p. 26). Experimentally, Astwood et al (1996) demonstrated the validity of this concept for some major food allergens relative to common non-food proteins using *in vitro* digestibility tests in simulated gastric fluid (SGF).

However, it is also recognized that some food allergens, such as those implicated in “oral allergy syndrome,” break down quickly in the human digestive system. As noted by J. M. Wal, “it is not always necessary to have intact protein in order to elicit food allergy” (Wal 1998, p. 421). Wal also cites research showing that denatured molecules and/or short peptide fragments can be as allergenic as the intact molecule, and that digestion of beta-lactoglobulin (a milk allergen) actually increases its allergenicity “by unmasking new epitopes which were hidden in the native spatial configuration of the molecule” (Ibid, p. 421). The EPA notes that one limitation of this test is that “it usually only tracks protein breakdown to fragments still recognized by the immunological reagents employed” (EPA BRAD 2000, IIB2). Thus, while digestive stability raises a red flag that makes further testing essential, lack of such stability cannot preclude the possibility that the protein is allergenic, and should not be used as a pretext to forego other tests.

## **6.2 How to measure digestive stability**

Both *in vivo* and *in vitro* test systems have been suggested, though simple *in vitro* tests are far more common in practice. A recent Scientific Advisory Panel recommends use of both methods as part of a decision-tree approach: “...the stability of introduced Bt-pesticidal gene products in the gastrointestinal tract should be tested by *in vitro* simulation of gastric and intestinal digestion and *in vivo*.” (SAP Bt Plant-Pesticides 2000, p. 75). According to this approach, a Bt protein shown to be stable *in vitro* would then undergo *in vivo* testing. Digestive stability *in vivo* would trigger further testing to determine bioavailability of the Bt protein and whether it is transferred to body fluids. While this provides a useful outline, it lacks the specificity needed to actually conduct tests. The crucial question of what precisely constitutes stability remains unanswered.

Test systems which attempt to mimic physiological conditions much more closely than simple *in vitro* tests in simulated gastric fluids (SGF) have also been proposed. For instance, several prominent experts have suggested a model developed by Minekus et al. (1995), which “simulates to a high degree the physiology of the stomach and small intestine of monogastric animals and man” (Helm 2001, p. 6; this system was also commended by Dr. Hubert Noteborn in SAP StarLink II Transcript 2000, pp. 399-400). This model takes account of factors such as temperature, pH, saliva, gastric and intestinal secretions (electrolytes, enzymes, co-factors, bile, and pancreatic juice), as well as gastric and intestinal mixing. “The model was developed as an alternative for human and animal experiments and validated successfully in comparison to *in vivo* experiments with human volunteers and fistulated pigs and calves for the digestion of proteins” (Helm 2001, p. 6).

## **6.3 Lack of standardization for *in vitro* digestive stability testing**

Whatever model is used, an operational definition of digestive stability requires a standardized test protocol. Widely varying experimental conditions make comparison of results from digestion tests on Bt proteins difficult. According to Taylor and Lehrer (1996): “Although the assessment of the resistance to hydrolysis of proteins could offer valuable information regarding the potential allergenicity of specific proteins, a rigorous protocol for such experiments has not been established... The development of further data on the comparative stability of food allergens vs. other food proteins to digestion, proteolysis, and hydrolysis would be highly

desirable to determine the ability of this tool for the assessment of the potential allergenicity of specific proteins.”

While further validation of this parameter is desirable, the fact that tens of millions of acres of genetically engineered crops are presently being cultivated – and in the case of Bt corn, consumed by millions of people – argues for prompt and rigorous application of the best scientific knowledge currently available with respect to this and other parameters of allergenicity.

#### **6.4 Towards a standardized protocol**

Since the EPA uses digestive stability as a criterion of the potential allergenicity of Bt proteins, the Agency should promptly establish and apply standardized protocols and evaluation criteria for registrants of Bt crops to follow in tests submitted to the Agency. Fortunately, there has recently been movement towards adoption of such a standardized test protocol.

Dr. Ricki Helm makes the following recommendations for standardization of parameters (Helm 2001, pp. 10-11):

- 1) Purity and activity of the enzyme employed;
- 2) Ratio of enzyme to test protein;
- 3) Use of digestible and non-digestible protein controls (bovine serum albumin and peanut allergen, respectively);
- 4) Assessment of both plant-produced and bacterial surrogate protein, isolated and in food matrix;
- 5) Range of pH values to account for higher values following a meal (1.0, 1.5, 2.0, 4.0 and 6.0);
- 6) Digestion sampling times (0, 15 and 30 seconds; 1, 2, 4, 8, 15 and 60 minutes)
- 7) Criteria of digestibility for acceptance
- 8) Temperature of the simulated gastric fluids (37°).
- 9) Standardized analytical methods for measuring degree of degradation (column chromatography [e.g. HPLC], SDS-PAGE, immunoblot analysis)

Even more valuable, a recent report by a panel of international allergy experts includes a detailed protocol for digestive stability testing (FAO/WHO 2001, pp. 12-13; see Appendix 2).

#### **6.5 Digestive stability of Monsanto's MON 810 corn**

The EPA concluded that the Cry1Ab protein expressed in Monsanto's MON 810 corn (derived from the HD-1 strain of *Bacillus thuringiensis kurstaki*) is not stable to digestion based on a single study conducted by the registrant (Monsanto Corn 1994). This study is seriously deficient in many respects, and is far from satisfying the criteria recommended above and by other experts.

- 1) Fragment, not full-length protein, tested: Monsanto failed to conduct its digestive stability test on the protein fragment expressed in its transgenic corn (apparently a 92 kD protein; see

Section 9.2); instead, the company employed the trypsin-resistant core of this Cry1Ab fragment (a 63 kD protein).

- 2) Core protein derived from bacterial surrogate, not plant: The trypsin-resistant core protein was not derived from the plant, but rather from *E. coli* transformed with the full-length Cry1Ab protoxin gene (not the gene fragment encoding the 92 kD fragment). The protoxin thus generated was subsequently treated with trypsin to obtain the trypsin-resistant core. The National Academy of Sciences (2000, p. 63), the SAP Bt Plant-Pesticides (2000, p. 73) and other experts recommend use of the plant-produced protein that people actually consume rather than a bacterial-produced surrogate for safety testing, because bacterial and plant-produced proteins can have significant differences. Alternately, both recombinant (e.g. *E. coli*-produced) and transgenic plant proteins should be tested (Helm 2001, p. 12; SAP Bt Plant-Pesticides, p. 75). The relevance of this issue was recently underscored by the SAP StarLink III, which roundly criticized antibody tests based on *E. coli*-derived Cry9C due to evidence that it differs from the plant-produced protein (SAP StarLink III, p. 32).
- 3) No matrix testing: Since the plant-produced protein was not tested in any form, it was not tested in its natural corn tissue matrix. Yet there is a growing consensus on the need to test both the isolated protein as well as the protein in its natural state as part of the relevant crop (SAP Bt Plant-Pesticides, p. 75; Helm, p. 7; FAO/WHO 2001, p. 12).
- 4) No controls: Monsanto failed to include any controls of known digestible or non-digestible proteins in its assay, making interpretation still more difficult.
- 5) Acidic pH: The protein was tested in simulated gastric fluid (SGF) at only one pH (= 1.2) near the acidic end of the range found in the human digestive system. Yet many experts now recommend that novel, genetically engineered proteins be tested in SGF at a pH of 2.0 (FAO/WHO, p. 13; see Appendix 2) or at a range of pH values (Helm, see criteria listed above). Speaking of digestive stability testing in general, Dr. Hubert Noteborn states: “The continual setting of the pH at a value of 1.2 does not mimic accurately the kinetics of the physiological events in the human stomach.” Noteborn goes on to describe a validated in vitro model of human digestion which shows pH values ranging from about 1.5 (fasting) to a maximum of between 5 and 6 soon (20 minutes) after a meal (SAP StarLink II Transcript 2000, p. 399). The EPA refused to approve StarLink’s Cry9C protein for human food use based in part on the results of a digestibility test performed at pH = 2.0 (Noteborn 1998). SAP StarLink III even refused to recommend approval of just 20 ppb Cry9C residues in foods based on this test and other evidence of potential allergenicity.
- 6) Very low test protein : pepsin ratio: Monsanto conducted its tests on an extremely small amount of protein, just 2 µg/ml of SGF containing 0.32% pepsin. This translates into an extremely low Cry1Ab : pepsin ratio of just 1 : 1600 by weight, or a molar ratio of approximately 1 : 3200. Other test protocols for digestive stability prescribe concentrations of test protein anywhere from 50 to 1,250 times greater than that employed by Monsanto.

## 6.6 Comparison to other digestive stability tests conducted on Cry1Ab

Fortunately, the digestive stability of Cry1Ab has been tested under different conditions by different researchers. Comparison of the results reveals huge variations, casting doubt on Monsanto's conclusion that Cry1Ab is not stable to digestion. These discrepancies may also provide clues as to the factors of most importance to digestive stability (Table 1).

The results shown in Table 1 vary dramatically depending on the respective test conditions. **While over 90% of the test protein was apparently degraded after just 2 minutes in the Monsanto study, it took at least two hours – 60 times longer – to achieve a comparable level of degradation in Noteborn's test system.** (The Aventis study showed intermediate results, with complete degradation within 15 minutes.) Both Aventis' and Noteborn's studies were aimed primarily at determining the digestive stability of Cry9C, the Bt protein expressed in StarLink corn, which was not approved for human consumption due in part to its pronounced stability to digestion. Cry1Ab(5) was included in each study for purposes of comparison. According to Noteborn:

“In comparison with Cry9C, the Cry-proteins Cry1Ab5 and CryIIIb showed also resistance to digestion in vitro whereas the enzyme PAT digested readily showing a complete digestion by the first time point of 2 minutes...” (Noteborn 1998, p. 21)

**Table 1: Comparison of digestive stability tests on Cry1Ab protein**

Study	Bt protein tested	End concentration of test protein in SGF*	pH	% degradation after given time periods in SGF	Western blot or SDS-PAGE: amount loaded per lane
Monsanto Corn (1994)	Trypsin-resistant core of Cry1Ab (63 kD) from full-length protoxin expressed in E. coli	2 µg/ml	1.2	20 sec.: 50% 2 min.: > 90%	10 ng (Western blot)
Aventis CropScience (2000a)	Cry1Ab expressed in recombinant E. coli	100 µg/ml	1.5	Complete degradation: With pepsin: < 15 min. W/o pepsin: 1 h	not determined
Noteborn (1998)	Cry1Ab5	165 µg/ml	2.0	2 min.: 59% 15 min.: 79% 30 min.: 79% 60 min.: 89% 2 hours: 91%	22.3 & 5.3 ug (2 batches) (SDS-PAGE)

\* SGF was prepared according to US Pharmacopoeia protocol in all three studies: Dissolve 2.0 g NaCl and 3.2 g pepsin in 7.0 ml hydrochloric acid and add sufficient water to make 1000 ml. Thus, pepsin concentration in SGF is 0.32% in all cases. Different normalities HCl were utilized to achieve different pH values.

Cry1Ab5 showed considerably more resistance to digestion than CryIIIb. While 41% of Cry1Ab remained intact at the 2-minute point, only 13% of CryIIIb remained undegraded. CryIIIb was completely degraded after 5 minutes, while 9% of intact Cry1Ab5 was found even after 2 hours of incubation in SGF (Ibid, Table 1, p. 31). Aventis reaches a similar conclusion:

“The Cry1Ab protein was digested at a similar, if slightly faster, rate than the *E. coli*-derived Cry9C protein in simulated gastric fluid.” (Aventis CropScience 2000, p. 17)

Several factors could account for the discrepancies between these three studies. I will focus mainly on the results obtained by Monsanto and Noteborn.

First of all, I have not been able to determine the precise nature of the Cry1Ab protein used by Noteborn. If it was the full protoxin, digestion could have been slowed by the additional degradation steps involved in breaking down this larger protein (about 130 kD) relative to the 63 kD fragment tested by Monsanto. Even if Noteborn’s Cry1Ab<sub>5</sub> is the toxic fragment, a minor structural variation peculiar to this particular Cry1Ab (quaternary rank “5”) could lend it more digestive stability than the trypsin-resistant core protein tested by Monsanto, though this seems unlikely. Different quaternary ranks are often assigned to identical proteins, and often indicate merely the particular study in which the protein was isolated.

A more likely factor involves the ratio of test protein to enzyme. Noteborn tests Cry1Ab<sub>5</sub> at a concentration over 80 times greater than that used by Monsanto (end concentration: 165 versus 2 µg/ml SGF). Monsanto’s 2 µg of test protein per ml SGF yields by far the lowest test protein : pepsin ratio I have seen in any test protocol (1 : 1600), whether actually employed or recommended. Clearly, Monsanto’s use of an extremely small quantity of protein relative to pepsin favors the most rapid possible digestion. The FAO/WHO study cited above recommends use of 2500 µg test protein per ml of SGF, a test protein : pepsin ratio of 1 : 1.3 (by weight) or a molar ratio of roughly 1 : 2.6 (see Appendix 2). Aventis used 100 µg/ml, Noteborn 165 µg/ml SGF.

Another factor that might explain the discrepancy between the results obtained by Monsanto and Noteborn is pH. Noteborn’s study employed a pH of 2.0, whereas Monsanto’s was conducted at pH = 1.2. It is not clear whether pH or concentration of the test protein (= test protein to pepsin ratio) is more important. Aventis, which used a pH value (1.5) and a test protein concentration (100 µg/ml SGF) that were both in between those used by Monsanto and Noteborn, also obtained intermediate results (degradation within 15 minutes).

Since a pH of 2.0 better represents the physiological pH range under a variety of conditions, and 165 µg/ml SGF better matches the concentration of test protein used in most digestive stability test protocols, Noteborn’s results should be given greater weight. Thus, one must conclude that Cry1Ab is at least somewhat resistant to digestion, contrary to Monsanto’s results.

In any case, it seems clear that the EPA should conduct a range of digestive stability tests – at various concentrations of test protein and a range of pH values – on the Bt protein produced in MON 810 corn. The considerations mentioned above should also be taken into account in the design of the experiment: use of the 92 kD protein rather than (or in addition to) the truncated 63 kD tryptic core; plant-produced protein rather than (or in addition to) the recombinant protein; the 92 kD plant-produced protein both in its isolated state and in its plant matrix; use of controls, etc.

### **6.7 Digestibility of Bt 11 corn protein**

Novartis Bt 11 protein was apparently not tested for digestive stability. The Monsanto study discussed above is cited as sufficient proof that the Bt protein expressed by Bt 11 corn, the product of a distinct transformation event, would behave in precisely the same manner as Monsanto's protein in simulated digestive fluid (EPA BRAD, p. IIB5). The Bt protein from this crop should also be tested properly for potential stability to digestion.

### **6.8 Digestibility of BollGard cotton protein**

The considerations discussed above for digestive stability testing on Monsanto's tryptic core HD-1 protein also apply to Monsanto's tests on its BollGard cotton protein (which is actually a hybrid protein composed of Cry1Ab and Cry1Ac – see Section 9.1). Examination of the test protocol reveals the same test conditions that proved so favorable to rapid digestion of the tryptic core Cry1Ab protein: 2 µg/ml SGF and pH = 1.2. And in fact, the test protein degraded in 2-7 minutes under these conditions (EPA BRAD, p. IIB12; Monsanto Cotton 1994c, pp. 14-15). Thus, the same need for more stringent digestibility tests applies to the BollGard cotton protein as well.

### **6.9 Digestibility of Cry1F protein**

Unfortunately, I did not have the opportunity to consult the registrant's digestive stability test on Cry1F, but must rely on the EPA's review (EPA BRAD Cry1F Corn, p. 10). Although the registrant used somewhat more protein (molar ratio of Cry1F : pepsin of 1 : 100, or roughly 1 : 50 by weight) than Monsanto, this still represents 2 to 3-fold less protein than was used by Aventis and Noteborn in the Cry1Ab(5) tests discussed above. Strangely, the EPA does not cite the pH at which this test was conducted, a crucial parameter. If it was done at pH = 1.2, the results – complete degradation in 5 minutes – are open to the same criticisms as were noted above for MON 810 corn and BollGard cotton.

## **7. Conclusion**

Table 2 summarizes the available data on the EPA's three parameters of allergenicity. For the Cry1Ab expressed by MON 810 and Bt 11 corn, there are two red flags (heat and digestive stability) and no information on amino acid homology. Reliable studies are lacking for all three parameters in the case of the Cry1Ab/Ac hybrid protein in BollGard cotton and the Cry3A protein in NewLeaf potato. The data are somewhat better for Cry1F corn, but the heat stability test is flawed by failure to measure the size of degradation products. The digestive stability test should have used a higher Cry1F : pepsin ratio and, if conducted at pH = 1.2, a higher pH.

It would be irresponsible of the Agency to re-register any of the Bt products without first collecting and evaluating *proper* studies on the three parameters discussed above. The case for suspension is particularly strong for the three varieties of Bt corn. First, the fact that Cry1Ab, the protein in MON 810 and Bt 11, exhibits digestive and thermal stability comparable to Cry9C, which is still a suspected allergen, is troubling. Secondly, transgenic varieties of corn utilizing Cry1Ab are grown on a wide scale, about 14 million acres. If Cry9C was able to contaminate up

to 22% of grain lots that were tested (Boston Globe 5/17/01), though StarLink was never grown on more than 0.4% of U.S. corn acreage (350,000 acres), then the Cry1Ab varieties, grown on 40 times more acreage, have been and are being consumed by virtually the entire American population. The EPA's failure to demand "required" information on even the basic (though insufficient) parameters of potential allergenicity 5 years after the products were first registered is completely unacceptable. Re-registration of these crops before these data have been collected and evaluated would be still more outrageous, making a mockery of the EPA's professed adherence to sound science in its regulation of these crops.

Yet there are still other reasons to doubt the adequacy of the Agency's health assessment. These issues relate to the EPA's reliance on a history of safe use of Bt microbial pesticides, deficient product characterization, the "bridging" of data between distinct corn lines, and the inadequate tests performed to determine whether bacterial-produced surrogate proteins are equivalent to their plant-produced counterparts for the purposes of safety testing.

**Table 2: Summary of Available Data for Human Health Assessment**

<b>Company Crop Bt protein</b>	<b>Digestive Stability</b>	<b>Heat Stability</b>	<b>Amino Acid Sequence Homology</b>
<b>Monsanto Yieldgard Corn Cry1Ab</b>	<b><u>RED FLAG</u></b> Digestive stability similar to that of StarLink Cry9C (1)	<b><u>RED FLAG</u></b> Heat stability comparable to that of StarLink Cry9C (2)	<b><u>NONE</u></b> (3)
<b>Syngenta Bt 11 Corn Cry1Ab</b>	<b><u>RED FLAG</u></b> Digestive stability similar to that of StarLink Cry9C (1)	<b><u>RED FLAG</u></b> Heat stability comparable to that of StarLink Cry9C” (2)	<b><u>NONE</u></b> (3)
<b>Monsanto BollGard Cotton Cry1Ab/Ac</b>	<b><u>INADEQUATE</u></b> Flawed study shows degradation in 2-7 minutes (4)	<b><u>INADEQUATE</u></b> Only shown to be “inactive” in processing study (5)	<b><u>NONE</u></b> (3)
<b>Mycogen &amp; Pioneer Herculex Corn Cry1F</b>	<b><u>RED FLAG</u></b> Test conditions not specified by EPA (6)	<b><u>INADEQUATE</u></b> Only shown to be “inactive” in bioassay after 30 min. at 75° & 90°C (5)	<b><u>OK</u></b> Though more stringent test would be desirable (7)
<b>Monsanto NewLeaf Potato Cry3A</b>	<b><u>RED FLAG</u></b> Test conditions not specified by EPA (5)	<b><u>NONE</u></b> (3)	<b><u>INADEQUATE</u></b> Additional data needed (8)

**Notes:**

- (1) “digested at a similar, if slightly faster, rate than ... Cry9C protein in SGF [simulated gastric fluids]” (Aventis CropScience 2000, “Cry9C Protein: The Digestibility of the Cry9C Protein by Simulated Gastric and Intestinal Fluids,” study submitted to the EPA, p. 17). See Section 6.6.
- (2) “relatively significant thermostability ... comparable to that of the Lys mutant Cry9C protein.” (Noteborn 1998, “Assessment of the Stability to Digestion and Bioavailability of the LYS Mutant Cry9C Protein from Bacillus thuringiensis serovar tolworthi,” study submitted to the EPA, p. 22). See Section 5.
- (3) “Amino acid homology comparisons for Cry1Ab, Cry1Ac and Cry3A against the database of known allergenic and toxic proteins were not submitted.” (EPA BRAD 2001, p. IIB2). See Section 4.
- (4) Monsanto conducted this study under conditions that proved extremely favorable to rapid digestion of the Cry1Ab/Ac hybrid protein: pH = 1.2, 2 µg test protein / ml SGF. Experts now recommend testing with higher concentrations of test protein at a milder pH (at least pH = 2.0). See Section 6.8.
- (5) “Inactive” here means “unable to kill insects” in bioassays, which provide little or no information about degradation of the protein into amino acids and small peptides, which is what should have been measured (e.g. by HPLC or SDS-PAGE). See Section 5.
- (6) EPA fails to cite the pH value of SGF. If test conducted at pH = 1.2, it should be repeated at pH = 2.0. See note (4) and Section 6.9.
- (7) Many experts recommend a more stringent test than one based on 8 contiguous amino acids. See Section 4.
- (8) EPA states that the data submitted by Monsanto are insufficient (EPA BRAD 2001, Table B1, p. IIB3).

## **8. History of Safe Use of Bt Microbial Pesticides**

In reviewing the potential health hazards of genetically engineered Bt crops, the EPA seems to place great weight on the “long history of safe use” of Bt microbial pesticides (EPA BRAD 2001, p. I3), even though this experience with Bt sprays is not cited as a formal criterion in its assessment. This loose reliance on a comparison with Bt pesticides is perhaps meant to compensate for the many inadequacies in the Agency’s formal assessment, as discussed above.

Yet there are a number of differences between Bt microbial pesticides and the Bt proteins expressed in plants that urge caution in the use of data generated with native Bt proteins as supportive of the registrations of Bt crops. Some of these differences are more relevant to non-target insect impacts and resistance development. Here, I will focus on those differences that have significance for the human health assessment.

First, the Bt proteins expressed in the three corn varieties being considered for re-registration are truncated versions of the Cry1Ab and Cry1F protoxins found in native Bt. Cry1Ab protoxin from the HD-1 strain of Bt is approximately 131 kD in weight, while Monsanto’s MON 810 corn apparently expresses a 92 kD modified fragment of the full-length HD-1 protein (Monsanto Corn 1995c, p. 15), and Novartis’ Bt11 produces a 69 kD fragment of the same (Northrup King Bt11 1994, p. 10). Mycogen/Pioneer’s Cry1F corn also expresses a truncated protein, 64 kD in weight (EPA BRAD Cry1F Corn 2001, pp. 5, 7). The protoxin is an insoluble crystalline protein that requires solubilization at an appropriate alkaline pH in the insect gut before it becomes active. The toxic fragments engineered into these two Bt corn varieties are apparently soluble and active.

Secondly, significant changes in codon usage were made to the native Bt genes to increase their expression in plants (see, for example, EPA BRAD 2001, p. IIA5).

Thirdly, there is evidence demonstrating that the toxic portion of Cry1A proteins can have a different three-dimensional structure depending on whether it is part of the protoxin or in its free state (Choma et al 1991), and that DNA structurally associated with the protoxin is released during the proteolysis process that generates the toxic fragment from the protoxin (Clairmont et al 1998). Since even slight changes in conformation can significantly impact a protein’s function and activity, including its susceptibility to proteolysis (Novartis Bt11, p. 18) and immunologic reactivity (Monsanto Corn 1995a, p. 91), this evidence would argue against over-reliance on data generated in tests on full-length Cry proteins (whether native Bt or E. coli-produced) in the assessment of the truncated plant versions found in Bt crops such as MON 810, Bt11 and Cry1F corn. Such a conformational difference might explain Monsanto’s finding that its full-length BollGard protein (a hybrid of Cry1Ab and Cry1Ac – see Section 9.1) has a lower antibody affinity than the core fragment of the same protein generated by treatment with trypsin (Monsanto Cotton 1994a, p. 55; Monsanto Cotton 1994b, p. 13). This finding might also explain the following cryptic observation made by the EPA in response to Monsanto’s request to use trypsin-resistant cores instead of full-length proteins in its safety assessments:

“The fact that variations to the ELISA results are attributed to different versions of the expressed endotoxin implies that the antibodies used for ELISA recognize a potentially blocked epitope.” (Monsanto Corn 1995a, p. 91)

This suggests that epitopes unavailable to antibodies in the structure of the full-length protein (BollGard cotton) or larger fragment (MON 810's 92 kD fragment) become exposed and available once proteolysis has converted the larger molecule to the trypsin-resistant toxic fragment.

Finally, to the extent that Bt protoxins *are* similar to their truncated toxins, one must point to the study by Bernstein et al (1999), which demonstrates that purified Cry protein extracts of Bt microbial pesticides containing Cry1Ab and Cry1Ac elicited IgE antibody responses in farm-workers exposed to them by the inhalant, dermal and possibly oral routes. As a result of this study, which was partially sponsored by the EPA, the Agency now has access to “...reliable Bt skin and serologic reagents...” in case it should ever decide to make use of them (Bernstein 1999, p. 581).

## **9. “Bridging” of Data in Assessment of Equivalence**

The question of whether it is appropriate to rely so heavily on data from Bt microbial pesticides, as the EPA does, in the assessment of Bt crop-produced proteins is complicated by the use of a third class of Bt proteins in these assessments – namely, proteins expressed in bacteria that have been transformed with the corresponding Bt gene sequences. This latter question of “test substance equivalence” will be addressed in more detail below (see Section 10). Here, I will discuss the complicated pathway by which registrants have attempted to demonstrate the safety of their plant-produced proteins with reference to their native Bt counterparts.

### ***9.1 BollGard Cotton***

The full-length Bt protein expressed in BollGard cotton is actually a hybrid molecule which is composed of amino acids 1 – 466 from Cry1Ab (from Btk HD-1) and amino acids 467-1178 from Cry1Ac (Btk HD-73) (Monsanto Cotton 1994a, p. 15). (Actually, it is important to keep in mind that the protein's primary structure has not been confirmed by sequencing, but is merely deduced from the nucleotide sequence.) This BollGard cotton hybrid protein differs by 7 amino acids from native Cry1Ac (Ibid, p. 15). Although Monsanto insists on designating it (mistakenly) as “Cry1Ac,” I will refer to it more precisely as “Cry1Ab/Ac hybrid.” This use of a hybrid molecule, combined with substitution of *E. coli* surrogate protein for the plant-produced version and other factors, makes it necessary for the registrant to construct four “bridges” from the protein actually produced in BollGard cotton to the native Cry1Ac protein found in the HD-73 strain of Bt. These bridging steps are outlined below, starting with the BollGard cotton protein (line 531), proceeding through the protein actually used to generate data in Monsanto's testing, and finally to the native microbial protein.

- 1) Line 531 to line 931: The company generates much of its data from IRC line 931 rather than the 531 line that was subsequently commercialized as BollGard cotton. Line 931 was chosen

for this purpose over 531 because it produces a higher level of the hybrid Cry1Ab/Ac protein in question. In fact, the line 531-derived protein was not even visible in a Western blot (Monsanto Cotton 1994a, p. 55). While the two lines apparently have the same nucleotide coding sequence, they do differ in “the random location of the gene insertion event.” (Monsanto Cotton 1994b, p. 13) If line 531 Cry1Ab protein was not even detected, it is hard to see how Monsanto could have evaluated any potential differences (beyond, perhaps, expression level) between 531 and 931-derived Cry1Ab proteins due to positional effects.

- 2) Line 931 to bacterial surrogate: Despite its higher level of expression, Monsanto decided that line 931 did not produce enough protein for the purpose of safety testing. Therefore, the company carried out an assessment to determine whether or not the line 931 Bt hybrid protein was equivalent to an E. coli-expressed surrogate with the same gene sequence (Monsanto Cotton 1994b). (This “test substance equivalence” study is discussed below.)
- 3) Bacterial surrogate to tryptic core: The bulk of Monsanto’s safety testing is not even conducted on the full length, E. coli-expressed, surrogate hybrid Cry1Ab/Ac, but rather on the “tryptic core” fragment generated from it by treatment with trypsin. This step involves cleaving the full-length 134 kD protein to produce the 66 kD trypsin-resistant core.
- 4) Tryptic core to native Bt: The final bridge constructed by the registrant was from the tryptic core of the E. coli-expressed surrogate hybrid Cry1Ab/Ac to the Cry1Ab and Cry1Ac proteins produced naturally in a strain (not necessarily HD-73) of Bt kurstaki (Monsanto Cotton 1994a, p. 16). As noted above, the native Cry1Ac differs by seven amino acids from the hybrid, six of them in the coding region for the toxic fragment. There is no explanation of why Monsanto constructed this hybrid protein for insertion into its BollGard cotton.

## **9.2 MON 810 Corn**

There is similar data bridging in the case of Monsanto’s MON 810 corn (EPA BRAD, p. IIA7). In this case, the original data were generated with ECB resistant corn line 754-10-1, which was used because it produces relatively high levels of the Btk HD-1 protein. These data were applied to another line of corn that produced lower levels of the protein in question, MON 801, which was subsequently commercialized (Monsanto Corn 1995a, p. 10). Finally, these data were used in support of the registration of MON 810 (Monsanto Corn 1995b), the line which is currently up for reregistration (MON 801 has been discontinued).

While line 754-10-1 and MON 801 expressed the full-length Cry1Ab protein encoded by a gene derived from Bt strain HD-1, MON 810 was spliced with a partial cry1Ab gene sequence that apparently encodes a 92 kD protein (Monsanto Corn 1995c, p. 15). This odd length matches neither the full-length Cry1Ab protoxin (131 kD) nor the toxic fragment. The explanation is that while Monsanto *attempted* to splice the gene coding for the full protoxin, its data suggest that only the first 2448 base pairs of the total 3468 base pair length of the gene were integrated into the corn genome.

“During the process of particle acceleration, the plasmid DNA can become broken resulting in the integration of partial genes into the genomic DNA.” (Monsanto Corn 1995c, p. 14).

“The full length 131 kD B.t.k. HD-1 protein was not observed in line MON 810, as expected, since the full length gene was not incorporated into the corn genome.” (Ibid, p. 15)

Thus, MON 810, the Bt corn being considered for reregistration, is the product of a transformation event gone awry.

Yet Monsanto did not conduct its test substance equivalence testing, nor its later health assessment tests, on this 92 kD fragment. In fact, the 92 kD fragment from the partially integrated cry1Ab gene was not even detected in Western blot assays, “probably due to low expression or rapid degradation to the trypsin-resistant product during the extraction procedure.” (Ibid, p. 15).

Instead, Monsanto employed the tryptic core fragment of the 92 kD protein, which has a molecular weight of approximately 63 kD. In fact, the registrant’s approach to bridging data between these three distinct corn lines, each the product of a separate transformation event, is to ignore the protein actually produced by each, and focus instead on the 63 kD fragment that results when the protein expressed by each of the three lines is treated with trypsin.

The final bridging step consisted in expressing the full-length Cry1Ab protoxin (not the 92 kD fragment apparently produced by MON 810) in *E. coli*, and likewise treating it with trypsin to obtain the *E. coli*-produced, tryptic core fragment.

### **9.3 Other Bt crops**

Much of the product characterization data for Cry1F corn also appears to derive from alternate corn lines (e.g. 1360 & 1362) rather than the line eventually commercialized (1507) (EPA BRAD 2001 Cry1F Corn 2001, pp. 5, 6). I have not had the opportunity to examine this matter in more detail for Cry1F corn or Cry3A potatoes.

### **9.4 Conclusion**

The permissibility of using alternate corn lines, the products of distinct transformation events, as the source of Bt proteins for testing purposes is unclear. The sources I have consulted do not address this issue, perhaps because they take it for granted that registrants will test the proteins from the corn line for which approval is sought. This is the straightforward path taken by Syngenta in the testing conducted on its Bt 11 line of corn (while Syngenta also employed protein expressed by *E. coli* in much of its safety testing, the company at least used the gene sequence from the corn line for which it sought approval to transform the bacterial surrogate; the issue of test substance equivalence in the strict sense is addressed below).

Likewise, I have seen no rational justification for permitting tests to be conducted on a derivative of the Bt protein that is actually expressed (whether in the plant or *E. coli*). Again, this is

probably because it is taken for granted that testing will be conducted on the protein as expressed, not a trypsinized or otherwise modified derivative of that protein.

Given the strict conditions for equivalence that many authorities in the field believe should be met before bacterial surrogates can be justifiably used for safety testing in lieu of plant-produced proteins, one would think that the sort of elaborate “bridging” of data between distinct corn lines and distinct proteins that Monsanto has engaged in would be frowned upon, to say the least.

## **10. “Test Substance Equivalence”**

Below, I will show that even the dubious data generated by Monsanto using alternate crop lines and protein derivatives do not meet the standards for test substance equivalence currently recommended by authorities in the field. According to the SAP Mammalian Toxicity 2000 (p. 14) and SAP Bt Plant-Pesticides 2000 (pp. 73-74), use of a bacterial-produced surrogate protein for testing purposes should be permitted only if it meets the following conditions for equivalence to its plant-produced counterpart:

- 1) Identical behavior of the full length as well as the trypsinated forms on 2D-gel electrophoresis;
- 2) Identical immunoreactivity (binding) to poly- and/or monoclonal antibodies;
- 3) Identical patterns of post-translational modification (i.e. glycosylation);
- 4) Sequence similarity of full length amino acid sequence (highly undesirable is the sequence analysis of 10-15 N and/or C-terminal amino acids and up to three short internal protein sequences).
- 5) Toxicity similarity to target herbivorous insect species (i.e. larvae).

### ***10.1 BollGard Cotton***

- 1) Monsanto ran a one-dimensional SDS-PAGE on full length and trypsinated forms of the Cry1Ab/Ac hybrid protein (Monsanto Cotton 1994b, p. 14), not a two-dimensional gel electrophoresis, as recommended. Based on this and the Western blot, the authors claim that major bands for both full length and trypsinated forms of the bacterial and plant proteins comigrate, but it is impossible to confirm this due to the poor quality of my copy of the gel. An anomalous 210 kD protein was found in several lanes of the Western blot (Ibid, pp. 16-17).
- 2) See 1) above.
- 3) The authors state that no carbohydrate staining was found at the Rf position of the native protein or the trypsin stable core. It is not clear if staining was found elsewhere (poor quality of copy). If so, such staining of other bands could mean that fragments of the protein are glycosylated, meaning failure to meet this requirement and possible allergenicity concerns.
- 4) The authors sequence only the 15 amino acids at the N-terminal of the trypsinated plant and E. coli proteins, together with the 15 AAs of a “minor signal” said to represent the native (untrypsinated) N-terminus of the E. coli protein, a method referred to as “highly undesirable” by the SAP. No internal protein sequences were analyzed. ***Only 8 of the 15 AAs of the***

*cotton-derived protein, 7 of the E. coli-expressed protein, and 8 of the untrypsinated “minor signal” were identified as conforming to the expected sequences.*

5) Insect bioassays appeared to confirm similar activity of plant and E. coli-derived bacteria.

Thus, even with questionable use of the tryptic core protein, the registrant met at best only 3 of the 5 conditions cited above as required for a determination of test substance equivalence (2, possibly 3, and 5). In particular, the authors have completely failed to demonstrate sequence equivalence of even the short N-terminal fragments being compared, with roughly half of the 15 amino acids remaining unidentified.

## **10.2 MON 810 Corn**

The bulk of Monsanto’s study to determine whether bacterial-produced surrogate protein was similar enough to substitute for the protein as produced in the plant was conducted on the original 754-10-1 line discussed above, which had been transformed so as to produce the full-length Cry1Ab protein derived from the HD-1 strain of Bt kurstaki rather than the 92 kD protein apparently produced in the MON 810 line that is up for reregistration. The surrogate protein was likewise the full-length 131 kD Cry1Ab protoxin expressed in transformed E. coli. Both protoxins – corn-produced and E. coli-derived – were then treated with trypsin to produce the 63 kD trypsin-resistant core fragments used in the equivalence tests (Monsanto Corn 1995a, p. 12).

Applying the same SAP Mammalian Toxicity criteria discussed above yields the following results:

- 1) Once again, Monsanto employed one dimensional SDS PAGE rather than the 2-D gel electrophoresis recommended by the SAP. The major bands appearing in plant and bacterial lanes comigrated, and were determined to weigh 59.8 kD, about 5% less than the theoretically expected 63 kD (Ibid, p. 18). Of perhaps more concern is the unmistakable presence of two strong minor bands in the bacterial surrogate lane, which appear to be 40 and 32 kD proteins. The 40 kD protein in particular appears to be nearly as strong as the main band. These bands are completely absent in the plant-produced protein lane. While 2 µg of bacterial-produced protein were loaded for this gel, the amount of plant-produced protein loaded is “not determined” (Ibid, Figure 3, p. 27; see Appendix 3).
- 2) The Western blot reveals major comigrating bands at about 56 kD for E. coli and plant-produced proteins. The E. coli protein lane, loaded with 20 ng, shows two minor bands, the stronger at about 34 kD and a considerably weaker one at about 38 kD. The corresponding plant-produced protein lane, whose load was not determined, reveals a single minor band at about 41 kD (Ibid, Figure 5, p. 29; see Appendix 4).
- 3) No evidence of glycosylation was found for either plant-produced or bacterial protein (Ibid, p. 19).
- 4) As with the cotton equivalence study discussed above, here too Monsanto employs the “highly undesirable” method of sequencing only the 15 amino acids at the N-terminal, with no internal sequencing whatsoever. 12 of the 15 amino acids of the E. coli protein conformed to the expected sequence; the amino acids in positions 13-15 could not be identified. 13 of 15 amino acids of the corn-produced protein were positively matched to the expected sequence,

with two tentative identifications at positions 1 and 13 (Ibid, pp. 17, 18 & Figures 1 & 2, p. 26).

- 5) Bioactivity of the two trypsin-resistant core proteins was tested on cotton earworm (CEW) and tobacco budworm (TBW). EC<sub>50</sub> values (the amount of protein required to inhibit growth by 50%) were reasonably close in each case, though the bacterial-produced surrogate proved to be somewhat more toxic than plant-produced protein to CEW, while somewhat less toxic than the plant protein to TBW. Thus, the ratio of EC<sub>50</sub> values (CEW/TBW) for the bacterial surrogate was 3.49, while the same parameter was only 2.07 for the tryptic core of the plant-expressed protein (Ibid, Table 1, p. 25).

Thus, even when the analysis is restricted to tryptic core fragments of the bacterial surrogate and plant-produced Cry1Ab proteins, MON 810 at best meets two (3 and 5) of the five criteria set forth by the SAP Mammalian Toxicity for justifying use of bacterial surrogate in place of the protein expressed in the plant.

## **11. EPA on the Use of Tryptic Core Proteins for Testing Purposes**

Monsanto sought permission from the EPA for its use of tryptic core fragment proteins in the testing of its Bt cotton and corn described above. The EPA's response deserves some consideration.

“In general the human safety of a full-length protein cannot be extrapolated from lack of toxicity of a smaller fragment. There is validity, however, in making the presumption that, for proteins in general, if the parent protein is known to be nontoxic to humans *and is a common dietary constituent*, then oral exposure to the fragments of the parent protein would not be toxic.

For the reasons forwarded by Monsanto, the tryptic fragment is appropriate as a test material for mammalian toxicity; and based on other information available to Monsanto, the full-length protein from B.t.k. HD-1 or HD-73 also is nontoxic” (Monsanto Corn 1995a, p. 91, my emphasis).

The Agency correctly notes that one *cannot* conclude that a full-length protein is safe based on testing conducted with a fragment of that protein. This would seem to invalidate the bulk of Monsanto's test results on its corn and cotton products, which were conducted on the trypsin-resistant core protein. In the next sentence, however, the EPA appears to open the window to extrapolation in the opposite direction: from the presumed safety of full-length microbial pesticide proteins (“parent protein”) to the truncated versions found in MON 810 and Bt 11 corn.

I have already outlined several reasons for not extrapolating from the history of safe use of native Bt microbial proteins to the safety of the truncated proteins expressed in most Bt crops. These include potential differences in solubility of full-length versus truncated Bt proteins, codon usage alterations for improved plant expression, evidence of conformational differences between full-length Bt proteins and their fragments, and differences in immunologic reactivity between full-length and truncated versions. In the case of the Cry1Ab/Ac hybrid protein expressed in BollGard cotton, we know that its *deduced* amino acid sequence differs by seven amino acids

from the native Cry1Ac produced by a strain, not necessarily HD-73, of *Bt kurstaki* (recall that the amino acid sequence itself has not been positively matched to the theoretical protein as deduced from its nucleotide sequence, beyond 7 or 8 amino acids at the N-terminal).

Additional evidence for the impermissibility of this extrapolation is provided by the Bernstein study discussed above, as well as a series of studies by Vazquez and colleagues demonstrating that Cry1Ac protoxin and toxin are potent immunogens that elicit both mucosal and systemic immune responses (Vazquez et al 2000a & 1999a), and that Cry1Ac protoxin is a potent systemic and mucosal adjuvant (Vazquez et al 1999b) and binds to surface proteins in the mouse small intestine (Vazquez et al 2000b).

In addition, the EPA presents no data that I am aware of demonstrating that native *Bt* proteins are in fact “a common dietary constituent,” as the Agency apparently assumes. Of course, *Bt* soil microbes expressing Cry1Ab and Cry1Ac proteins are present in the environment, and it may be assumed that they are present – at some undetermined level and frequency – on crops. Yet without data to demonstrate the prevalence of such proteins on food crops, it would seem unjustified to consider them “a common dietary constituent.” Another source of exposure is microbial pesticides applied in the form of *Bt* sprays. Yet when applied externally, the Cry proteins in *Bt* sprays degrade rapidly in ultraviolet light. It is unclear if either or both of these contributions to human Cry protein exposure justify the assumption that native Cry1Ab and Cry1Ac are commonly found in the human diet. In any case, it is safe to assume that Cry proteins expressed within corn varieties planted on 14 million acres result in much higher levels of exposure than rapidly degradable *Bt* sprays applied sporadically at best to crop plants.

Until the EPA addresses the issues discussed above, it would seem irresponsible to rely on data on the history of safe use of the full-length protoxins expressed in microbial pesticides to justify any conclusion as regards the safety of the truncated and otherwise modified versions of these proteins expressed in crop plants. On the other hand, the EPA itself disallows conclusions concerning the “human safety of a full-length protein” from “lack of toxicity of a smaller fragment.”

Finally, the Agency explicitly designated its response to Monsanto concerning use of tryptic core fragments in the health assessment of *Bt* crops as “interim guidance” (Monsanto Corn 1995a, p. 87). Now, over half a decade later, the Agency should conduct a thorough re-appraisal of this provisional guidance in light of the most current data as part of the re-assessment process, and postpone any decision on re-registration of any *Bt* crop until this is done.

## **12. Concluding Thoughts**

Based on the many and glaring deficiencies in the EPA’s product characterization and human health assessment discussed above, it appears that the Agency too often regards its assessment responsibilities as a mechanical collection of data to “confirm” a foregone conclusion (e.g. “complete a product database”) rather than as a vital process to ensure protection of public health and the environment. Numerous additional examples of this false and corroding attitude are revealed in the Agency’s language. With respect to the *in vitro* digestibility assay, the Agency

writes: “The intent of this assay is to demonstrate that the Bt protein is degraded into small peptides or amino acids in solutions that mimic digestive fluids” (EPA BRAD Human Health, p. IIB1). Yet if this assay is to mean anything at all, its intent cannot be to “demonstrate” a foregone conclusion (here, degradation into small units). Rather, the purpose would have to be to “*determine whether or not* the Bt protein is degraded...” As we have seen, Monsanto appears to have deliberately crafted its digestibility test on the tryptic core of the bacterial-produced surrogate of the protoxin expressed in its line 754-10-1 corn (as a proxy for the Bt protein produced in the line up for reregistration, MON 810) to arrive at the conclusion it desired. This is not sound science, and the Agency should not adopt the practices and attitude of the registrant, which obviously has a financial interest in the outcome of such testing.

Another example. With respect to determining whether bacterial-derived Bt proteins are similar enough to plant-produced proteins to justify substitution of the former for the latter in testing, the EPA writes: “Proper characterization of the equivalency between these microbial proteins and plant expressed proteins provides an alternative to purifying the test material as the plant-produced protein from large volumes of tissue.” (EPA BRAD Product Characterization, IIA4). Once again, the language employed (“proper characterization *of the equivalency*”) implicitly prejudices the outcome of testing that obviously could fail to demonstrate the presumed conclusion (here, “equivalency” of plant-produced and surrogate bacterial proteins). Once again, the Agency appears to adopt the attitude of the biotech industry, which commonly refers to such studies with language such as “determination of test substance equivalence,” even when, as in the case of Cry9C, the study in fact fails to demonstrate equivalence. The fact that Aventis’ failure to demonstrate such “equivalency” was a key factor leading SAP StarLink III to advise against the company’s petition for a tolerance for Cry9C demonstrates that this is by no means a case of hairsplitting.

A prominent allergy expert tells us that:

“The incidence of food allergy is increasing and the severity of the symptoms commonly observed is worsening. ... Food allergy has long been considered by some as fanciful. However, it is now a real and particularly serious aspect of food toxicology.

“The number of incriminated food allergens is also increasing. Novel and traditional foodstuffs appear to be potential allergens. This is a major concern for both the food industry and those regulatory committees in charge of public health. There is therefore a need to assess and manage the allergenic risk of (new) foods.” (Wal 1998, p. 413)

The EPA should take seriously the possibility that Bt crops have contributed to the rising incidence of food allergy. In terms of regulation, this implies a rigorous application of the best science available rather than flaccid acceptance of whatever shoddy studies the financially interested registrant companies choose to submit. With respect to this re-assessment process, the Agency should not even consider re-registration until it has collected the data it lacks, generated new data where necessary in line with the rigorous testing recommended by the Agency’s own expert advisors, and fully evaluated the same. Anything less would be to put the interests of the registrants above the overriding concern for human health.

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