Genetically Engineered Crop Health Impacts Evaluation - GAPS Analysis

Prepared by Bill Freese, Research Analyst, Friends of the Earth
July 2003 (updated October 2003)

It seems a simple question: "Are genetically engineered foods harmful to human health?" The purveyors of sound-bite science have an equally simple and satisfying string of answers: "No, not at all. Genetic engineering is precise. These foods are thoroughly tested. The regulatory agencies vouch for their safety."

But take a closer look and these simple answers fall apart.

In fact, genetic engineering is a haphazard process, more of an art than a science because it lacks repeatability, and results in many more abortions than successes. With rare exceptions, the transgenic proteins actually produced in these foods have not been tested at all, providing no answer regarding their health impacts, if any. And contrary to popular opinion, the Food and Drug Administration (FDA) has ***not*** approved ***any*** GE food as safe.

In the following GAPS analysis, we delve deeply into some of the most important concerns about GMOs rather than cite every single study suggesting potential health impacts. Another feature that sets this review apart is reference to material that is largely or completely unknown to the scientific community (e.g. unpublished studies submitted to the EPA by Monsanto, FDA consultation documents). This GAPS analysis is broken down into three parts:

1) Gaps in the U.S. GE foods "regulatory" system;
2) Glaring inadequacies of the testing regimes ***as practiced;*** and
3) Case study of Bt corn

Gaps in the U.S. GE Foods "Regulatory" System

Regulation or Rubber Stamp?

Genetically engineered (GE) food "regulation" in the U.S. is based on the dogma of substantial equivalence – the extremely strong presumption that neither the genetic transformation process nor the foreign gene construct or protein will impair the wholesomeness of the transgenic crop[1]. Think about this for a moment. The regulatory system is founded on the notion that GE foods are unchanged, hence safe, and so do not require testing or regulation[2]. All the weaknesses of the system flow from this paradoxical assumption.

This explains why:

1) The FDA's consultation process is ***voluntary*** rather than mandatory[3];
2) FDA never examines the original studies conducted by companies, but rather only the company's summary assessment of its own research;
3) Companies can and do deny FDA requests for additional data, and FDA misses obvious errors in data summaries that a thorough review would have uncovered[4]; and finally,
4) The FDA merely conveys the company's conclusion as to the food's "substantial equivalence," pointedly avoiding any sort of explicit approval of its own. This is perhaps due to liability concerns on the part of the government.

In Europe, on the other hand, "substantial equivalence" is hypothetically assumed only as a ***starting point*** for investigation[5]. A particular GE crop may very well not be substantially different than its conventional counterpart, say European scientists, but first we must subject them to an in-depth examination to confirm or deny this hypothesis.

Another reason for the lack of meaningful regulation in the U.S. is the enormous influence the biotechnology industry, particularly the Monsanto

[1] The FDA steps in only when there is glaring reason to think substantial equivalence does not apply – i.e. the transgenic protein comes from a known allergenic source, something which all companies avoid anyway, especially since it was demonstrated in 1996 that a soybean spliced with a Brazil nut gene elicited skin prick reactions in Brazil-nut allergic people, as well as IgE binding of their sera.

[2] In fact, GE foods "regulation" was introduced as a "de-regulatory" initiative by the Bush Senior administration. See "Biotechnology Food: From the Lab to a Debacle," New York Times, Jan 25, 2001 for a revealing look at how the U.S. "regulatory" system for GE foods was developed, as told by industry and government officials.

[3] A good example of the political rather than scientific nature of GE foods regulation is the FDA's recent decision to shelve long-standing plans to make consultations mandatory. The Bush Administration wanted to avoid any hint that U.S. regulation of GE foods is deficient while the WTO challenge of European Union GE foods regulation is underway.

[4] "Holes in the Biotech Safety Net: FDA Policy Does Not Assure the Safety of Genetically Engineered Foods," by Doug Gurian-Sherman, Center for Science in the Public Interest, January 2003; pp. 4-7.

[5] Kuiper et al (2001). "Assessment of the food safety issues related to genetically modified foods," The Plant Journal 27(6), p. 504.

Corporation, has had in writing the rules. According to an important *New York Times* article on this subject (see footnote 2):

> *"What Monsanto wished for from Washington, Monsanto and, by extension, the biotechnology industry got. If the company's strategy demanded regulations, rules favored by the industry were adopted. And when the company abruptly decided that it needed to throw off the regulations and speed its foods to market, the White House quickly ushered through an unusually generous policy of self-policing.*
>
> *Even longtime Washington hands said that the control this nascent industry exerted over its own regulatory destiny through the Environmental Protection Agency, the Agriculture Department and ultimately the Food and Drug Administration was astonishing.*
>
> *"In this area, the U.S. government agencies have done exactly what big agribusiness has asked them to do and told them to do," said Dr. Henry Miller, a senior research fellow at the Hoover Institution, who was responsible for biotechnology issues at the Food and Drug Administration from 1979 to 1994."*

This testimony – from government and biotech industry sources – makes other claims regarding the undue influence of the biotech industry on GE food issues more credible. For instance, there is evidence to suggest that Monsanto initiated the chain of events leading to the dismissal and discrediting of Dr. Arpad Pustzai, whose animal research suggested that potatoes engineered to produce lectins (which are similar in nature to the Bt toxins in GE pesticidal crops) could be responsible for causing gastric lesions.[6]

Obstacles to independent evaluation of GE crops:

Despite numerous calls by scientists for more independent research into the potential health and environmental impacts of GE foods, the U.S. government allocates shamefully little money to this end. The U.S. Department of Agriculture, for instance, spends just $3.6 million out of a $193 million research budget to support studies that examine potential environmental impacts. Even when independent researchers are funded, a finding of potential harm requiring follow-up can effectively disqualify those scientists from additional funding. For instance, one scientist found suggestive evidence that the insecticidal proteins found in Bt spray and Bt crops could be allergenic in a study approvingly cited and reviewed by expert advisers to the Environmental Protection Agency (EPA). He has been unable to obtain funding for further research in this area. Another scientist has done EPA-sponsored research on unintended effects in Bt corn, as well as the environmental impacts of Bt

[6] "The Sinister Sacking of the World's Leading GM Expert – and the Trail that Leads to Tony Blair and the White House," by Andrew Rowell, The Daily Mail (UK), July 7, 2003

insecticidal proteins. He, too, has had difficulty obtaining funds to continue these lines of research (source: personal communications).

Other scientists have been unable to obtain the GE crop for independent animal feeding studies. One example is a Japanese scientist who was denied access to modest amounts of DuPont's high-oleic soybeans by both DuPont and the Japanese government when that crop was being reviewed by Japanese regulatory authorities (source: personal communication).

Still other researchers have obtained permission to study GE crops only after agreeing to onerous restrictions. For instance, one common condition forced on scientists is a pledge not to sequence the transgenic protein. Ironically, full sequencing of the transgenic protein has long been recommended by numerous expert bodies as a basic prerequisite for a sound evaluation, and to the best of our knowledge, this information is never supplied by companies[7].

In fact, even prestigious government review bodies have been denied access to basic information required for sound reviews of these crops owing to excessive claims of "confidential business information."[8]

As a result, there is hardly ever any independent research available to confirm or dispute the GE crop developer's claims of safety. And as we shall see, even when such research is available, U.S. regulators tend to ignore it, preferring to base their evaluations solely on company-provided information.

Flaws in reviews of GE crops that appear to demonstrate safety:

One source of confusion on the potential health impacts of genetically engineered foods is the tendency of many expert scientific bodies to issue reports that are inherently contradictory. (Examples include committees of the National Academy of Sciences and the U.K. Royal Society). That is, they often call for more stringent testing regimens ***and*** state (or imply) that currently marketed GE crops are safe – which of course begs the question of how inadequately tested crops can be judged safe. The purveyors of sound-bite science have made a cottage industry of publicizing the latter claims while ignoring the serious criticisms of current testing regimens made by the very same bodies.

Often, the contradiction is only apparent. The expert body will say that there is no evidence that GE foods on the market are unsafe. Yet "lack of evidence" often reflects the lack of adequate studies – absence of evidence rather than evidence of absence.

---

[7] Normally, only the 10-25 amino acids at the N-terminal of the transgenic protein are sequenced.

[8] See, for example: "Environmental Effects of Transgenic Plants: The Scope and Adequacy of Regulation," Committee on Environmental Impacts Associated with Commercialization of Transgenic Plants of the National Research Council, National Academy of Sciences (2002), pp. 11, 177. http://books.nap.edu/catalog/10258.html.

A related error is to make an unjustifiable distinction between currently marketed GE crops, which are said to be safe because they have "simple" and well-understood modifications, and future applications, which because of their greater complexity will require more robust testing regimens[9]. While stacked crops, for instance, may in some cases pose greater risks than those with single-transgene traits, there is no scientific basis for distinguishing the two categories with respect to stringency of testing required. This is especially true in the arena of unintended effects, which can be triggered by the genetic transformation process *per se* (or by widely used viral promoter sequences) rather than the particular transgene(s) introduced.

A third common thread in the numerous expert reviews is their reliance on the opinions of national regulatory agencies, particularly the FDA, which as noted above are themselves based on "data summaries" from the financially interested biotech companies rather than original studies. Even if members of such expert bodies want to examine the original studies, they often either cannot gain access to this sensitive material (considered proprietary, see footnote 8) or simply do not have time to examine those studies that may be available, relying instead on selective summaries of these studies by the regulatory agencies (e.g. Scientific Advisory Panels to the EPA, source: personal communication).

A fourth consideration is conflict of interest. The expert bodies are often comprised mainly of plant science specialists who themselves receive research funding from biotechnology companies, or whose institutions receive such funding.

To take just one example, in a 2002 report on the potential health impacts of genetically engineered crops[10], the pro-biotech U.K Royal Society called on the U.K. Food Standards Agency to "consider whether post-marketing surveillance should be part of the overall safety strategy for allergies, especially of high-risk groups such as infants and individuals in 'atopic' families." The Royal Society also concedes that ***the current criteria for human health assessments of GE crops are neither explicit nor objective.*** Given these and other criticisms, the report's familiar statement that "There is at present no evidence that GE foods cause allergic reactions" rings hollow, yet this is the misleading message most often conveyed to the press and even the wider scientific community. A second example is detailed in the following section.

[9] For one example, see: G. J. Persley, "New Genetics, Food and Agriculture: Scientific Discoveries - Societal Dilemmas," for The International Council of Science, June 2003.

[10] "Genetically modified plants for food use and human health – an update," The Royal Society, February 2002. Available at http://www.royalsoc.ac.uk/templates/search/websearch.cfm?mainpage=/policy/cur_gm.htm.

Inadequacies of the testing regimes *as practiced*

Four especially troubling issues are detailed below. The list is not exhaustive. Please note that the treatment below, unlike most critiques of this sort, deals with specific examples of commercialized or field-tested GE crops. Sources include difficult-to-obtain, unpublished corporate studies and other documents, such as Scientific Advisory Panel reports to the EPA, that are mostly unknown to the scientific community.

Surrogate proteins:

Biotech companies almost never test the transgenic protein actually produced in their engineered crops. Instead, for testing purposes they make use of a bacterial-generated ***surrogate*** protein that may differ in important respects from the plant-produced one. The same genetic construct used to transform the plant is spliced into ***bacteria*** (usually *E. coli*), and these bacteria are grown out. The surrogate transgenic protein is then extracted from the bacteria, and sometimes processed (e.g. cleaved with trypsin to generate its "tryptic core"). This bacterial-derived surrogate protein (or its derivative) is then employed for all subsequent testing: short-term animal feeding studies, allergenicity assessments, etc.

Several scientists to whom we described this practice expressed amazement. They take it for granted that plant and bacteria will generate different transgenic proteins from the same gene, even if transformed with the very same genetic construct. Testing a surrogate, they say, is no substitute for testing the real thing. This is because:

1) The foreign DNA actually integrated into the plant genome will differ from that taken up as a plasmid by bacteria due to the peculiarities of each transformation "event" (as the name implies, each "event" is unique and non-repeatable); for instance, it is not uncommon to find that only fragments of the intended gene have been incorporated into the plant's genome; disruption of native DNA often occurs adjacent to the site(s) of insertion.

2) Even if precisely the same foreign DNA is incorporated into bacteria and plants, the two organisms – which are kingdoms apart in biological terms – generate and process proteins differently. For instance, most bacteria do not add sugar molecules to proteins, while plants do, in a process known as glycosylation. Plant glycosylation patterns present the risk of immune responses, including allergic reactions.

As a result, animal feeding studies and allergenicity assessments that make use of bacterial surrogate proteins or their derivatives may not reflect the

toxicity or allergenicity of the plant-produced transgenic protein to which people are actually exposed.

Biotech companies use surrogate proteins for testing purposes because they find it inconvenient to extract sufficient quantities of transgenic proteins from their plants. Yet several expert bodies on both sides of the Atlantic have criticized this practice. To take just one example, according to a National Academy of Sciences committee that conducted an exhaustive review of Bt crops: "Tests should preferably be conducted with the protein as produced in the plant." If surrogates are nonetheless used:

> *"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."*[11]

Three years later, the EPA has still failed to do this, even though its scientific advisers have proposed such "test substance equivalence" criteria.[12] In fact, the toxicity and allergenicity assessments of Bt crops currently on the market employed surrogate proteins that did ***not*** meet these criteria[13]. The same is true of most or all non-Bt engineered crops as well.

This is not an academic point. The StarLink Scientific Advisory Panel – comprising some of the nation's leading allergists – strongly criticized the FDA for using such a bacterial surrogate Cry9C (rather than StarLink Cry9C) in its allergy assay: "The use of non-equivalent, bacteria-derived coating antigen raises the possibility that IgE directed against plant derived Cry9C may not be detected" (which would mean false negatives). For this and other reasons: "The test, as conducted, does not eliminate StarLink Cry9C as a potential cause of allergic symptoms."[14]

In fact, the advisors cautioned that ***any*** level of StarLink in food might be harmful:

---

[11] "Genetically Modified Pest-Protected Plants: Science and Regulation," Committee on Genetically Modified Pest-Protected Plants, National Research Council, National Academy of Sciences, 2000, p. 65, see: http://books.nap.edu/catalog/9795.html. For similar recommendations, and examples of immunologic differences between nearly identical proteins, see: "The StarLink Affair," Friends of the Earth, July 2001, sections 9.2 to 9.4, at www.foe.org/safefood/starlink.pdf.

[12] "Mammalian Toxicity Assessment Guidelines for Protein Plant Pesticides," EPA's Scientific Advisory Panel, SAP Report No. 2000-03B, Sept. 28, 2000, p. 14. http://www.epa.gov/scipoly/sap/2000/june/finbtmamtox.pdf.

[13] Freese, B. (2001), "A Critique of the EPA's Decision to Reregister Bt Crops and an Examination of the Potential Allergenicity of Bt Proteins," adapted from comments of Friends of the Earth to the EPA, Dec. 9, 2001. Available at: www.foe.org/safefood/comments.pdf.

[14] "Assessment of Additional Scientific Information Concerning StarLink Corn," EPA's Scientific Advisory Panel, SAP Report No. 2001-09, pp. 29-30. http://www.epa.gov/oscpmont/sap/2001/july/julyfinal.pdf.

"... the Panel concluded that based on reasonable scientific certainty, there is no identifiable maximum level of Cry9C protein that can be suggested that would not provoke an allergic response and thus would not be harmful to the public." (p. 35)

Given the use of bacterially produced surrogate proteins as the norm, one cannot avoid the conclusion that the plant produced transgenic proteins we actually eat in our food are virtually untested.

Unintended effects:

Artificial introduction of foreign genetic constructs into plants creates numerous opportunities for potentially hazardous unintended effects, which include over-production of native allergens or toxins, nutritional deficits, creation of novel fusion proteins (i.e. proteins from inadvertent combination of plant and foreign DNA in the transformation process) with unknown properties, and horizontal transfer of transgenic DNA (including antibiotic resistance markers) to bacteria residing in the human gut. As the regulatory system was being designed in the early 1990s, FDA scientists called for GE crop-specific regulations to test for such "pleiotropic" effects. But they were overruled by administrative superiors, who insisted on a "deregulatory" system that permitted biotech companies to bring their novel GE crops to market as cheaply as possible, meaning no mandatory testing or even review.[15]

Unintended effects are common. Some – especially blatant effects – are caught and weeded out during the development process. Subtle effects may remain undetected for years after commercialization. David Schubert, professor of cell biology at the Salk Institute, reports that engineering a human gene into human cells has been shown to significantly increase or decrease the expression levels of fully 5% of the genes in the cell (as measured by mRNA levels.)[16] The same is likely true of engineered plants, though no regulatory agency requires or applies techniques to detect such changes.

Some phenomena likely to cause unintended effects, such as horizontal gene transfer, were once dismissed as all but impossible. However, recent evidence from what has been called the first human GE food feeding trial demonstrates that the herbicide resistance gene in glyphosate-resistant soybeans is indeed transferred to, ***and expressed in***, human gut bacteria.[17] There is no reason to think that antibiotic resistance marker genes used in GE crops may not also

[15] See www.bio-integrity.org/list.html for internal memos from FDA scientists concerning the inadequacy of the regulatory framework proposed and adopted in 1992. See also "Biotechnology Food: From the Lab to a Debacle," by Kurt Eichenwald, Gina Kolata and Melody Petersen, New York Times, 1/25/01 for a revealing look at how biotech firms influenced development of the U.S. regulatory framework.

[16] For one of many references, see: "A different perspective on GM food," by David Schubert, professor of cell biology at the Salk Institute, Nature Biotechnology, Vol. 20, October 2002.

[17] Netherwood T, Martin-Orue SM, O'Donnell AG, Gockling S, Gilbert HJ and Mathers JC. Transgenes in genetically modified Soya survive passage through the small bowel but are completely degraded in the colon. Study conducted for the UK Food Standards Agency, July 2002.

transfer to gut bacteria, and from there through conjugation to other, perhaps pathogenic, bacteria. This finding has strengthened long-standing concerns on the part of the British Medical Association and many others that GE crops might promote the spread of antibiotic-resistant, pathogenic bacteria and so impair the efficacy of these drugs.

In 2002, the National Academy of Sciences convened a panel to consider unintended, health-related effects of plant genetic engineering, and the means to detect them. (The very fact that this panel was convened validates the decade-old concerns of FDA working scientists.) European scientists advocate non-targeted techniques for measuring the levels of hundreds of proteins, metabolites, and/or messenger RNAs to increase the chances of detecting unintended effects,[18] as does Dr. Schubert (footnote 16). Monsanto, for some reason, opposes this approach,[19] which means that U.S. regulators will most likely not even recommend its use. In the U.S., regulators generally see nothing but summary data from companies on gross compositional analyses (i.e. fat, protein and starch levels) together with targeted screening of a handful of compounds (e.g. amino acids). However, there are no data requirements; companies submit summaries of whatever research they choose to conduct.

Visual inspection, or the "gross abnormality" test:

The case of barnase:

Barnase is an enzyme that degrades single-stranded RNA molecules. A bacterial form of barnase is a known toxin, causing kidney damage when perfused into rats.[20] A bacteria-derived version of barnase is spliced into corn and other crops to induce male sterility, which it does by rendering the anthers incapable of producing viable pollen grains. For example, the barnase gene has been engineered into Aventis' MS6 line of male-sterile corn,[21] which was deregulated by USDA for commercial cultivation in 1999. It is linked to a promoter fragment from an "anther-specific" gene, which is designed to limit expression of the toxin to anther tissue. However, it is well-known that so-called tissue-specific promoters drive production of low levels of transgenic protein in non-target tissues. Thus, more careful scientists refer to them as "tissue-preferred" promoters rather than "tissue-specific," admitting that "some expression may occur in other parts of the plant."[22]

---

[18] Kuiper et al (2001), op. cit.

[19] Roy Fuchs of Monsanto, Power Point presentation to the NAS committee cited above.

[20] Ilinskaya and Vamvakas (1997). "Nephrotic effect of bacterial ribonucleases in the isolated and perfused rat kidney," Toxicology 120, pp. 55-63.

[21] FDA's Consultation Note on Aventis' Male-Sterile Corn, *MS6* Line, April 4, 2000. See Memo for BNF No. 66 at http://www.cfsan.fda.gov/~lrd/biocon.html. (Note: FDA issues an extremely brief document – a "note to the file" – for genetically engineered crops that are the subject of "voluntary consultations" between the FDA and the developer. The note to the file [normally about 4 pages of 1 ½-space text] merely conveys some basic facts about the crop and the developer's assurances that it is substantially equivalent to the conventional crop.)

[22] For example, see: "Commercial production of aprotinin in plants," U.S. Patent 5,824,870 awarded to Baszczynski et al.

Because of its toxicity to the rat kidney, barnase could present food or feed safety concerns if expressed in corn kernels or fodder. How did Aventis test for possible expression of barnase in its MS6 corn? According to the FDA, Aventis: 1) Assumed that any level of barnase expression in tissues other than the anther would result in "abnormal plant growth"; 2) Not observing abnormal plant growth, Aventis concluded that barnase was not present anywhere else in the corn plant. There is no analysis of the assumption that any level of barnase will entail abnormal plant growth. Nor is there any discussion of the potential human toxicity of bacteria-derived barnase in corn kernels, despite its troubling mechanism of action (nucleic acid-degrading) and nephrotic effects in rats.

In any case, visual inspection is obviously not the best method for detecting a toxin in a food crop. Aventis should have performed ELISAs or similar protein detection assays to detect any barnase present in kernels and other non-anther tissues.

Interestingly, a 2002 patent on male sterile plants granted to the very same company (Aventis) frankly admits that "expression of the sterility DNA (e.g. barnase DNA) in tissues other than the stamen cells, e.g., in cells during tissue culture or in somatic cells of the plants or seeds" can occur. ***In fact, one of the chief aims of the patent is "to counteract the undesired effects of possible low level expression of the male-sterility gene (e.g. comprising the barnase DNA)"***[23] through co-engineering barstar, a barnase inhibitor, into the plant.

What are these "undesired effects"? We are not told, but Aventis was surely aware of them in 1999, when the USDA cleared MS6 male-sterile corn for commercial cultivation. Despite its recognition that even low levels of barnase can have "undesired effects," Aventis brought MS6 corn to market without even testing corn kernels or other tissues for the presence of barnase. USDA deregulated MS6 for commercial cultivation in 1999, and FDA issued its consultation memo in 2000, without such data.

One final note. Aventis ***did*** perform an ELISA to detect the phosphinothricin acetyltransferase enzyme (PAT) that is co-engineered into the MS6 line along with barnase. PAT lends resistance to the herbicide glufosinate, has been widely used in genetic engineering, and is generally considered safe. Why did Aventis take the trouble to assay for the likely innocuous PAT and neglect to do the same for a known toxin? One possible explanation is that the company realized that barnase would be found in corn kernels, and that this would raise food safety concerns that it preferred not to deal with. Does Aventis male-sterile corn pose a health risk to consumers? We don't know, and neither do

[23] Michiels et al. "Method to obtain male sterile plants," U.S. Patent 6,344,602 awarded to Aventis CropScience, February 5, 2002.

the FDA or Aventis. "Don't look, don't find" is a common strategy in both industry and regulatory circles.

The case of viral-vectored trichosanthin:

In 1991, 1996 and perhaps subsequent years, the USDA approved open-air field trials of tobacco engineered to produce an extremely toxic compound – trichosanthin – derived from the roots of a Chinese plant. Trichosanthin belongs to the class of ribosomal inhibitor proteins (RIPs), which operate by inactivating a cell's protein-making machinery (i.e. ribosomes). It is similar to two other members of this group – ricin and abrin – that are among the most toxic substances known to man. It is an extremely potent RIP, able to inhibit protein synthesis by 50% in an assay involving young rabbit blood cells at a concentration of just 0.1 ng/ml.[24] Trichosanthin has a long history of use in China to induce abortions. Effects associated with the intravenous use of trichosanthin include toxicity to embryos and fetuses,[25] renal toxicity,[26] neurological disorders,[27] fever, headache, arthralgia and skin rashes.[28]

The tobacco plants were infected with a tobacco mosaic virus (TMV) that had been transformed with the trichosanthin gene. TMV is known to infect tomatoes, peppers, eggplant, potatoes and other tobacco relatives in the Solanaceous family. Thus, these trials obviously raised food safety concerns.

In its environmental assessment of the 1991 trial[29], the USDA made three key assumptions on the basis of little or no evidence:

1) Low level: The level of trichosanthin in the infected tobacco "should be below any significant level of biological activity;"

2) No contamination: Tobacco plants would die if high levels of trichosanthin were generated, thus limiting spread of the trichosanthin-bearing virus to conventional tobacco and related food crops;

---

[24] Kumagai et al (1993). "Rapid, high-level expression of biologically active α-trichosanthin in transfected plants by an RNA viral carrier," Proc. Natl. Acad. Sci., Vol. 90, p. 430.

[25] Chan et al (1993). ""Developmental toxicity and teratogenicity of trichosanthin, a ribosome-inactivating protein, in mice," Teratog Carcinog Mutagen 1993, 13(2), pp. 47-57.

[26] Ko & Tam (1994). "Renal reabsorption of trichosanthin and the effect on GFR," Renal Failure 16(3), pp. 359-66.

[27] Kahn et al (1990). "The safety and pharmacokinetics of GLQ223 in subjects with AIDS and AIDS-related complex: a phase I study," AIDS 4(12), pp. 1289-91.

[28] Dharmananda, Subhuti, Ph.D., Director, Institute for Traditional Medicine, Portland, Oregon. "Trichosanthines." See www.itmonline.org/arts/tricho.htm.

[29] "Environmental Assessment and Finding of No Significant Impact" for Permit No. 91-007-08 granted to Biosource Genetics for a field trial conducted in North Carolina in 1991. See: http://www.isb.vt.edu/biomon/relea/9100708r.eaa. No EAs are available for the 1996 or any subsequent trials. See Appendix 4 of "Manufacturing Drugs and Chemicals in Crops: Biopharming Poses New Threats to Consumers, Farmers, Food Companies and the Environment," by Bill Freese for Friends of the Earth (2002) for a detailed examination of viral-vectored trichosanthin. Available at: www.foe.org/biopharm/.

3) No human health impact: Trichosanthin would have no human health impacts upon oral ingestion, based in part on assumptions 1 (low expression level) and 2 (low potential for contamination of food crops) above. Dermal and inhalant exposure were not even considered.

These three assumptions all proved to be wrong:

1) High level: An experiment conducted around the same time with this same system demonstrated that TMV-vectored trichosanthin was generated at a level of 2% of total soluble protein in tobacco, at that time ***"the highest accumulation of a foreign protein ever reported in any genetically engineered plant;"*** [30]

2) Potential for contamination: Despite this high level of expression, there was no indication that the tobacco plants were killed, contrary to the USDA's assumption: "The viral symptoms consisted of plant stunting with mild chlorosis and distortion of systemic leaves…"[31]

3) Possible health impacts upon ingestion: In 2001, Health Canada (Canada's FDA) issued a warning against ***ingestion*** of a Chinese medication containing "trichosanthin alkaloid, which is known to cause mutations in human cells and malformations in embryos, suppress the immune system, and produce severe allergic reactions. The safe and effective dose of this herb is not known."[32]

In both cases – barnase and trichosanthin – biotech companies and federal regulators failed to assess transgenic crops for their potential to expose consumers to known toxins because they relied strictly on visual inspection and irresponsible assumptions.

Failure to establish/follow test protocols:

There are very few established protocols for assessing the potential human health impacts of GE crops. Instead, one finds loose guidelines that in most cases only recommend certain tests or procedures without specifying how they are to be conducted. Allergenicity test guidelines are an important case in point. Since 1996, various groups have devised so-called "decision trees" that lay out a series of tests (e.g. structural similarity to known allergens, digestive and heat stability, sera screening, etc.) to assess the potential allergenicity of transgenic crop proteins.[33]

[30] Kumagai et al, op. cit., p. 429, my emphasis.
[31] Ibid, p. 429.
[32] "Health Canada Warns Consumers About Chinese Medications," Health Canada press release, Feb. 28, 2001. See www.acupuncture.com/herbology/chest-relief2.htm.
[33] For instance, see: Metcalfe et al (1996). "Assessment of the Allergenic Potential of Foods Derived from Genetically Engineered Crop Plants," Critical Reviews in Food Science and Nutrition 36(S), pp. S165-186.

Until a 2001 report by an FAO-WHO expert consultation[34], however, none of these decision-trees specified test conditions. As a result, biotech companies have been free to devise procedures of their own choosing – procedures that have invariably yielded negative results. Still worse, regulators have failed to collect ***any*** studies on some of these important parameters in the case of most Bt crops. In one particularly egregious case, the EPA even ignored a 1998 study by an FDA scientist indicating the potential allergenicity of the transgenic protein in most Bt corn, and instead requested that the financially interested developer (Monsanto) submit its own analysis, without specifying test conditions, by March 15, 2003.[35] (See case study below.)

The broader scientific community, including even some scientists who have reviewed GE crops for allergenicity, are largely unaware of these facts. Perhaps due to the repeated assurances of the public spokespeople for federal regulatory agencies about the supposed viability of the regulatory process, they incorrectly assume that currently marketed GE crops have passed stringent reviews for allergenicity.

As we shall see, if evaluated according to the detailed 2001 FAO-WHO allergenicity test protocol cited above, most currently registered Bt corn would not pass muster.

[34] "Evaluation of Allergenicity of Genetically Modified Foods," Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, Jan. 22-25-2001. www.fao.org/es/esn/food/pdf/allergygm.pdf.

[35] "Biopesticides Registration Action Document: Bt Plant-Incorporated Protectants – Overview," Environmental Protection Agency, October 15, 2001. http://www.epa.gov/pesticides/biopesticides/pips/bt_brad2/1-overview.pdf

Case Study – Bt Corn

Our concerns about Bt corn derive from four sources:
1) Suggestive evidence of allergenicity from human and animal studies as well as allergen-like properties of the Bt insecticidal protein Cry1Ab;
2) Unintended consequences of the genetic engineering process;
3) Regulatory failure; and
4) Differences between insecticidal proteins in Bt sprays and Bt crops.

Bt sprays versus Bt crops:

Bacillus thuringiensis (Bt) is a soil microbe that produces a variety of insecticidal crystalline proteins. Preparations of Bt spores are widely used in spray form by organic and conventional farmers to control certain pests. Most Bt corn varieties are engineered to generate modified versions of Cry1Ab, one of the major insecticidal proteins found in Bt sprays. There has been next to no independent testing of Bt corn and other Bt crops for potential human health impacts. However, even the very few studies conducted on the related Bt sprays raise concerns about the potential allergenicity of Bt corn. We will first briefly examine the evidence from Bt spray studies. At the end of this case study, we will examine similarities and differences between the insecticidal proteins in Bt sprays versus Bt crops to gain a better idea of how these data apply.

Suggestive evidence of allergenicity from:

Human studies

Allergic symptoms including allergic rhinitis, angioedema, dermatitis, pruritus, swelling, erythema with conjunctival injection, exacerbations of asthma, angioedema and rash have been reported in farmworkers and others exposed to Bt spraying operations.[36]

Bernstein et al (1999) demonstrated that purified Cry protein extracts of Bt microbial pesticides containing Cry1Ab and Cry1Ac elicited positive skin tests and IgE antibody responses in two farmworkers exposed to them by the inhalant, dermal and possibly oral routes. Positive skin tests and the presence of IgE antibodies in serum are considered indicators of allergenicity. Though Bernstein did not observe allergic reactions in these workers, he notes that they were tested after only 1 to 4 months of exposure, and that "clinical symptoms would not be anticipated unless there was repeated long-term exposure…" In addition, he notes that the "healthy worker effect" might have skewed his results – that is, susceptible farmworkers might have associated

[36] See references 6-8 in Bernstein et al (1999), "Immune responses in farm workers after exposure to Bacillus thuringiensis pesticides," Environmental Health Perspectives, 107(7): pp. 575-582.

their allergic symptoms with Bt, sought other employment to avoid exposure, and hence not have been included in his study (see study cited in footnote 36).

Animal studies

Additional evidence is provided by Vazquez and colleagues in a series of studies demonstrating that Cry1Ac protoxin[37] and toxin are potent immunogens that elicit both mucosal and systemic immune responses,[38] and that Cry1Ac protoxin is a systemic and mucosal adjuvant similar in potency to cholera toxin.[39] They also found that Cry1Ac binds to surface proteins in the mouse small intestine.[40] It should be noted that Cry1Ac is very similar in structure to the Cry1Ab insecticidal protein in most varieties of Bt corn.

In an assessment of Bt crops[41], expert advisors to the Environmental Protection Agency (EPA) who reviewed the Bernstein study and one of Vazquez et al's four studies concluded that:

> *"These two studies suggest that Bt proteins could act as antigenic and allergenic sources." (p. 76)*

Different approaches – including post-market surveillance – are called for to further characterize the allergenic risk of Bt proteins:

> *"With respect to allergenicity, the Panel concluded there is a continuing need to explore further approaches whereby the potency of allergic reactions of [sic] the isolated Cry-pesticidal protein and the transgenic plant can be more comprehensively assessed." (p. 75)*
>
> *"Only surveillance and clinical assessment of exposed individuals will confirm the allergenicity of Bt products or for any other novel protein introduced into the diet of consumers." (p. 76).*

Finally, the EPA's experts note that testing for potential reactions to Cry proteins in Bt spray and Bt crops could be undertaken now:

---

[37] Protoxin = inactive precursor protein that yields the insecticidally active toxin upon cleavage.

[38] Vazquez et al (1999a). "Intragastric and intraperitoneal administration of Cry1Ac protoxin from Bacillus thuringiensis induces systemic and mucosal antibody responses in mice," Life Sciences, Vol. 64, No. 21, pp. 1897-1912; Vazquez et al (2000a). "Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from Bacillus thuringiensis HD 73 in mice," Brazilian Journal of Medical and Biological Research 33: pp. 147-155.

[39] Vazquez et al (1999b). "Bacillus thuringiensis Cry1Ac protoxin is a potent systemic and mucosal adjuvant," Scandinavian Journal of Immunology 49, pp. 578-584.

[40] Vazquez et al (2000b). "Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine," Biochemical and Biophysical Research Communications 271, pp. 54-58.

[41] SAP Bt Plant-Pesticides (2000). "Bt Plant-Pesticides Risk and Benefit Assessments," FIFRA Scientific Advisory Panel Report No. 2000-07, March 12, 2001. http://www.epa.gov/scipoly/sap/2000/october/octoberfinal.pdf.

> *"The importance of this [Bernstein's] report is that reagents are available that could be used for reliable skin testing and serological evaluation of Bt protein exposed individuals." (p. 76)*

Unfortunately, in 2001 the EPA re-registered Bt corn for 7 years without making use of these reagents. The Agency also ignored other evidence of the potential allergenicity of Cry proteins in Bt crops.

Similarities to known allergens:

The versions of Cry1Ab protein found in hybrids derived from the two major Bt corn events (Monsanto's MON810 and Syngenta's Bt11) exhibit at least three properties considered characteristic of food allergen proteins by leading experts: structural similarity to known allergens, digestive stability and heat stability.

Structural similarity: All allergenicity testing protocols require that the structure of the novel, transgenic protein be compared to those of known allergens. Matching sequences of 6 to 8 amino acids (depending on the protocol) raise a red flag necessitating further testing. Food and Drug Administration scientist Steven Gendel demonstrated amino acid homology between several Cry proteins and known food allergens. Gendel found that Cry3A (Bt potatoes) and β-lactoglobulin, a milk allergen, shared sequences 7-10 amino acids in length. He also identified sequences of 9-12 amino acids shared by Cry1Ab (Bt corn) and vitellogenin, an egg yolk allergen. Gendel concluded that:

> *"…the similarity between Cry1A(b) and vitellogenin (Fig. 4) might be sufficient to warrant additional evaluation."*[42] *(p. 60)*

The EPA failed to collect any amino acid homology studies from Monsanto prior to the product's original registration in 1996, or even upon its re-registration in 2001.

Digestive stability: Many food allergens are stable to digestion. It is thought that the longer a protein survives in the gut, the more likely it is to induce the cascade of immune system events leading to allergic sensitization and reaction in susceptible individuals. Most food proteins – both native and transgenic – break down rapidly in the gut due to the action of protein-degrading enzymes and acid. Novel proteins (or rather, their bacterial surrogates) are normally tested *in vitro* in acidic solutions containing pepsin. The rate of breakdown is significantly influenced by the amount of pepsin relative to test protein in, and the acidity of, the "simulated gastric fluid."

[42] Gendel, S. (1998). "The use of amino acid sequence alignments to assess potential allergenicity of proteins used in genetically modified foods," Advances in Food and Nutrition Research 42, pp. 45-62.

Two digestive stability studies[43] on Cry1Ab by Bt protein expert Dr. Hubert Noteborn established that:

1) After 30-180 minutes in simulated gastric fluid (SGF), 9-21% of Cry1Ab remains undigested;
2) After two hours in SGF, Cry1Ab degrades only to fragments of substantial size at the low end of the range considered typical of food allergens (15 kilodaltons);
3) Cry1Ab is substantially more resistant to digestion than 4 other transgenic proteins tested (including one other Cry protein, Cry3A); of the six proteins tested, only StarLink corn's Cry9C exhibited greater digestive stability.

Aventis CropScience also found that Cry9C and Cry1Ab possessed similar digestive stability:

> *"The Cry1Ab protein was digested at a similar, if slightly faster, rate than the E. coli-derived Cry9C protein in simulated gastric fluid."[44] (p. 17)*

In contrast, Monsanto's digestive stability test on Cry1Ab employed highly acidic conditions (pH 1.2) and a huge excess of pepsin relative to test protein – conditions that favor the most rapid possible digestion[45]. Thus, it's no surprise that Monsanto's results (over 90% degradation in just 2 minutes) vary by a factor of 60 from those of Hubert Noteborn (cited above). Dr. Noteborn found that 10% of Cry1Ab survived for 1-2 hours, not 2 minutes. Under the authoritative allergenicity testing protocol recommended by international experts at FAO/WHO and accepted widely by national regulators outside the U.S., Cry1Ab would show itself to be still more stable than in Noteborn's test.

Heat stability: Dr. Noteborn also found that Cry1Ab possessed "relatively significant thermostability … comparable to that of the Lys mutant Cry9C protein" found in StarLink corn.[46] The EPA failed to collect any heat stability studies from Monsanto.

The similarities discussed above are summarized in Appendix 1. The EPA's lack of response to these studies is discussed below (Regulatory Failure).

---

[43] Noteborn, H. (1998). "Assessment of the Stability to Digestion and Bioavailability of the LYS Mutant Cry9C Protein from Bacillus thuringiensis serovar tolworthi," submitted to the EPA by AgrEvo, EPA MRID No. 447343-05 (Cry1Ab was also tested for purposes of comparison); Noteborn et al (1995). "Safety assessment of the *Bacillus thuringiensis* insecticidal crystal protein CRYIA(b) expressed in transgenic tomatoes," in Engel, et al (eds.), American Chemical Society Symposium Series 605, Washington, DC, pp. 134-47.

[44] Byard, J. (2000). "Cry9C protein: The digestibility of the Cry9C protein by simulated gastric and intestinal fluids," submitted to the EPA by Aventis CropScience. EPA MRID No. 451144-01.

[45] Ream, J.E. (1994). "Assessment of the *In vitro* Digestive Fate of *Bacillus thuringiensis* subsp. *kurstaki* HD-1 Protein," unpublished study submitted to the EPA by Monsanto, EPA MRID No. 434392-01.

[46] Noteborn (1998), op. cit., p. 22.

Unintended consequences of the genetic engineering process:

Fragmented and uncharacterized fusion protein in MON810

Many Bt corn hybrids planted on millions of acres in the U.S. are derived from Monsanto's MON810 "event," which contains the Cry1Ab insecticidal toxin discussed above. However, Monsanto's unpublished molecular characterization study on MON810[47] reveals that the genetic construct broke apart during the transformation process, resulting in several unintended consequences whose implications have not been adequately assessed (or acknowledged) even now, 7 years after market introduction:

1) Only a gene fragment (about 70%) of the intended full-length cry1Ab protoxin gene was incorporated into MON810;

2) As a result, the NOS termination sequence was not incorporated; instead, the cry1Ab gene fragment fused with adjoining corn DNA;

3) Monsanto scientists were unable to detect the putative 92 kD fusion protein presumably generated by the fused cry1Ab gene fragment and corn DNA; tests on the corn apparently revealed only the 63 kD "tryptic core" protein that Monsanto presumes to be a breakdown product of the fusion protein.

None of Monsanto's safety testing was conducted on the putative 92 kD fusion protein produced by MON810 (which was undetectable). Thus, its properties remain unknown. Even worse, the bacterial surrogate protein (see Surrogate Protein above) used by Monsanto for testing purposes was not even the same size as that produced by MON810. Monsanto generated a full-length 131 kD version of Cry1Ab in *E. coli*, extracted it, then treated it with trypsin to generate the 63 kD active fragment. Results of testing with this bacterial surrogate "tryptic core protein" may not reflect the toxic and allergenic profile of the putative corn-produced fusion protein[48].

The EPA glossed over the potential implications of this failed transformation process in its review of MON810 corn. Because it relied on confusing and/or incomplete summary information provided by Monsanto, ***the FDA was apparently not even aware that MON810 contained a gene fragment and***

[47] Levine et al (1995). "Molecular Characterization of Insect Protected Corn Line MON 810," unpublished study submitted to the EPA by Monsanto, EPA MRID No. 436655-01C.

[48] Lee et al (1995). "Assessment of the Equivalence of the *Bacillus thuringiensis* subspecies *kurstaki* HD-1 Protein Produced in *Escherichia coli* and European Corn Borer Resistant Corn," unpublished study submitted to the EPA by Monsanto, EPA MRID No. 435332-04; Lee and Bailey (1995). "Assessment of the Equivalence of *B.t.k.* HD-1 Protein Produced in Several Insect Protected Corn Lines and *Escherichia* coli," unpublished study submitted to the EPA by Monsanto, EPA MRID No. 436655-03. Contrary to their titles, these studies did ***not*** demonstrate equivalence between bacterial surrogate and corn-produced Cry1Ab according to criteria recommended by the EPA's advisers (see reference in "Surrogate Proteins" section above). See also: Freese, B. (2001). "A Critique of the EPA's Decision to Reregister Bt Crops and an Examination of the Potential Allergenicity of Bt Proteins," adapted from comments of Friends of the Earth to the EPA, Dec. 9, 2001. Available at: www.foe.org/safefood/comments.pdf.

produced a fusion protein.[49] Appendix 2 details the fundamental errors in the FDA's consultation document on MON810. The regulatory failures with respect to MON810 are addressed more fully below.

Increased lignin - failure to detect/follow up on a striking unintended effect

Bt corn hybrids derived from Monsanto's MON810 and Syngenta's Bt11 events exhibit increased levels of lignin in stem tissue[50]. This finding accords with anecdotal reports from farmers that Bt corn is stiffer and less desirable to farm animals as fodder (lignin is the woody component of plants and is non-digestible).

Lignin is the product of three aromatic compounds – coniferyl alcohol, p-coumaryl alcohol and sinapyl alcohol – all of which are derived from phenylalanine, an essential aromatic amino acid. Phenylalanine, in turn, is a product of the shikimic acid pathway, which is reportedly responsible for generating compounds comprising 35% and more of the dry mass of higher plants[51]. The discovery of increased lignin levels in Bt corn raises the question of whether other intermediates and products associated with the lignin and shikimic acid biosynthetic pathways have been affected by the transformation process. Aromatic biomolecules are extremely important in both plants and mammals as building blocks for hormones and other bioactive substances. The limited testing for a handful of compounds undertaken by Monsanto and Syngenta might easily have missed unintended increases or decreases in the levels of these other bioactive substances.

Finally, the finding that two completely different transformation events (MON810 and Bt11) are associated with increased lignin levels raises an interesting question. Normally, one would expect that each non-repeatable, unique transformation "event" would yield unique unintended effects related to the site of insertion (i.e. interruption, up- or down-regulation of a native gene(s)), scrambling of plant DNA adjacent to the site of insertion, number of (fragmented) copies of the gene that were introduced, or other factors unique to the event. Finding the very same unintended effect from two different transformation events suggests that the genetic transformation process *per se* (here, particle bombardment) might be responsible for an increase in lignin levels, and perhaps other, yet undetected, effects. Why was the increased lignin content of Bt corn brought to light only 5 years after market introduction? Why hasn't targeted testing been conducted for other bioactive

[49] The author has pointed out these serious errors at an FDA scientific forum and personally to James Maryanski, head of biotech at FDA. To my knowledge, neither Mr. Maryanski nor anyone else at FDA has followed up on this matter.

[50] Saxena and Stotzky (2001). "Bt Corn Has a Higher Lignin Content than Non-Bt Corn," American Journal of Botany 88(9), pp. 1704-1706.

[51] Alibhai and Stallings (2001). "Closing down on glyphosate inhibition – with a new structure for drug discovery," Proceedings of the National Academy of Sciences, Vol. 98, No. 6, pp. 2944-46.

substances associated with the lignin and shikimic acid pathways? Why haven't non-targeted techniques such as metabolic profiling been applied? Why are we asking these questions only now rather than 7 years ago? All these unanswered questions represent gaps in the human health assessment of Bt corn.

Similarities and differences between Bt crops and sprays:

The EPA's chief justification for approval of Bt crops in the absence of crucial data is that Bt sprays have a history of safe use, and so Bt crops are presumed to be safe as well. This presumption is not justified for several reasons. First of all, it is reasonably clear that Bt sprays do cause allergic symptoms, as detailed at the start of the case study above. Expert advisers to the EPA have advised the Agency that more studies are needed to determine the allergenic risk posed by Cry proteins in general – whether from Bt sprays or crops. Secondly, there is likely much greater exposure to Cry proteins in Bt crops than in sprays. Cry proteins in Bt sprays break down quickly upon exposure to sunlight, while this is obviously not the case with Bt crops, which produce the toxin internally in most or all plant tissues, including grain. Thirdly, Bt sprays are composed of bacterial spores comprised chiefly of Cry protoxins – the inactive precursors of the insecticidal Cry toxins. These protoxins become active toxins upon cleavage under alkaline conditions obtaining in the guts of certain insects. Bt crops, on the other hand, are generally engineered to produce the Bt toxin, which is active without processing. There is also evidence indicating that Cry toxins are more immunoreactive than Cry protoxins.[52]

Even if one ignores the evidence that Cry proteins from Bt sprays are likely allergenic, it is completely unacceptable to conclude that Bt crops are safe due to the absence of testing of the plant-derived proteins.

Breakdown in the regulatory system:

The question of whether Bt corn hybrids are harmful to consumers is still open. Testing along the lines indicated above is urgently needed to answer it. However, even if proper testing were to prove them to be safe, this case study dramatically illustrates the fundamental flaws in our "de-regulatory" system for genetically engineered crops. Consider the following:

1) The EPA approved Monsanto's Bt corn, MON810, with virtually no consideration of the potential implications of the failed transformation event leading to generation of a putative (because undetectable) 92 kD fusion protein;

2) The EPA approved MON810 on the basis of studies that employed a derivative of a surrogate bacterial protein rather than the plant-produced

[52] See Freese, B (2001), op. cit., Section 8.

fusion protein; studies purporting to demonstrate the equivalence of the surrogate and fusion proteins for testing purposes did not meet standards recommended by experts;

3) The EPA registered and re-registered (in 2001) MON810 without making any effort to follow up on suggestive evidence of allergenicity. In particular, the EPA ignored an important study by an FDA scientist showing structural similarity to a known food allergen, did not require submission of a heat stability study, and accepted a rigged digestive stability study.

4) The FDA's consultation document on MON810 contains fundamental errors regarding the basic molecular features of the transgenic protein, despite the fact that the pertinent study was available at its sister agency, the EPA. Once these errors were pointed out, the FDA apparently made no effort to follow-up;

5) There has likewise been no effort to investigate the potential health implications of a marked unintended effect of the engineering process – namely, increased lignin levels in Bt corn stalks, suggesting that the levels of lignin or related compounds could be altered in other corn tissues.

The case of MON810 is not exceptional. It illustrates not just that the U.S. regulatory system has holes that need fixing. Rather, it shows that the system is not about food safety at all, but rather was designed to speed transgenic crops to market as quickly as possible on the strong ***a priori*** presumption of no human health impacts. That there is any shell of a regulatory system in place at all in the U.S. has more to do with the perceived need by industry and government to reassure a rightly concerned public that these foods have received the government's stamp of approval.[53]

[53] See "Biotechnology Food: From the Lab to a Debacle," NYT, cited in footnote 2.

Appendix 1: Allergenicity Assessment of Bt Crops

In October of 2001, the EPA re-registered the entire class of Bt crops: 3 varieties of corn and one of cotton (potatoes were originally given an unlimited registration). The Agency was supposed to undertake a thorough-going reassessment, taking account of the most current scientific information and the recommendations of its scientific advisors, prior to making a decision. As detailed below, the EPA not only failed to do this, but did not even collect the most basic information needed to conduct an allergenicity assessment of the Cry1Ab, Cry1Ac/Ab, Cry1F and Cry3A crop varieties.

The following table outlines key deficiencies in the EPA's assessment. The three parameters are those chosen by the EPA (EPA BRAD Human Health Assessment). The notes following the table provide references for those wishing to explore this matter further. The table is excerpted from a study by Friends of the Earth, available at: www.foe.org/safefood/comments.pdf.

Summary of Available Data for Human Health Assessment

Company Crop Bt protein	**Digestive Stability**	**Heat Stability**	**Amino Acid Sequence Homology**
Monsanto Yieldgard Corn Cry1Ab	**RED FLAG** Digestive stability similar to (though lesser than) that of StarLink Cry9C (1)	**RED FLAG** Heat stability comparable to that of StarLink Cry9C (2)	**RED FLAG** Matches found with vitellogenin, an egg yolk allergen, over 9-12 amino acid-length subsequences (3)
Syngenta Bt 11 Corn Cry1Ab	**RED FLAG** Digestive stability similar to (though lesser than) that of StarLink Cry9C (1)	**RED FLAG** Heat stability comparable to that of StarLink Cry9C (2)	**RED FLAG** Matches found with vitellogenin, an egg yolk allergen, over 9-12 amino acid-length subsequences (3)
Monsanto BollGard Cotton Cry1Ab/Ac	**INADEQUATE** Flawed study shows degradation in 2-7 minutes (4)	**INADEQUATE** Only shown to be "inactive" in processing study (5)	**RED FLAG** Cry1Ab/Ac has the same vitellogenin-matching subsequences as Cry1Ab in the pertinent region (3, 6)
Mycogen & Pioneer Herculex Corn Cry1F	**INADEQUATE** Test conditions not specified by EPA (7)	**INADEQUATE** Only shown to be "inactive" in bioassay after 30 min. at 75° & 90°C (5)	**OK** Though more stringent test would be desirable (8)
Monsanto NewLeaf Potato Cry3A	**INADEQUATE** Test conditions not specified by EPA (7)	**NONE** (9)	**RED FLAG** Amino acid sequences found in which 7-10 matched β-lacto-globulin, a milk allergen (10)

Notes to Human Health Assessment Table

(1) "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, "Cry9C Protein: The Digestibility of the Cry9C Protein by Simulated Gastric and Intestinal Fluids," study submitted to the EPA by Aventis CropScience, p. 17). In another study, Noteborn (1998) found that it took two hours to achieve > 90% degradation of Cry1Ab(5) in SGF (165 µg/ml SGF, pH = 2.0) Noteborn (1998), p. 21, Annex 1 – Table 1, p. 31. See note (2) for full Noteborn citation.

(2) "Studying the Cry1Ab5 protein a relatively significant thermostability was observed which was 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 by AgrEvo, p. 22)

(3) "…the initial alignment between Cry1A(b) and vitellogenin located subsequences in which 9 to 11 amino acids were identical (82% similarity). Realignment indicated that these regions contained stretches of 11 biochemically similar and 12 evolutionarily similar amino acids (100% similarity over 11 or 12 amino acids." "For example, the similarity between Cry1A(b) and vitellogenin might be sufficient to warrant additional evaluation." Gendel, Steven M. "The use of amino acid sequence alignments to assess potential allergenicity of proteins used in genetically modified foods," Adv. in Food and Nutrition Research , Vol. 42, 1998, pp. 58-60. The EPA apparently did not consider this study in its reassessment of Cry1Ab corn. The Agency states merely that companies did not submit structural comparisons: "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)

(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 much higher concentrations of test protein at a milder (at least pH = 2.0).

(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)

(6) "Cry1A(c) has the same sequence as Cry1A(b) in the region involved, and therefore produced the same alignments, but this was not considered an independent alignment because the proteins are closely related." Gendel, Steve, p. 59. (See note (3) for citation)

(7) 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).

(8) Many experts recommend a more stringent test than one based on 8 contiguous amino acids.

(9) "No heat stability studies were available for Cry3A." EPA BRAD 2001, p. IIB2.

(10) "First, the initial alignment between Cry3A and β-lactoglobulin located subsequences in which 7 of 10 amino acids matched exactly. Realignment with both the evolutionary and biochemical matrices indicated that the intercalary amino acids were similar, meaning that the alignment was 100% similar over 10 amino acids." Gendel, Steve, pp. 58-59. See note (3) for citation. The EPA apparently did not consider this study in its reassessment of Bt crops, stating merely that "additional amino acid sequence homology" data are needed to "complete product database" for Cry3A NewLeaf potatoes. EPA BRAD 2001, Table B1, p. IIB3.

Appendix 2

A Sampling of Errors in the FDA's "Note to the File" for Monsanto's Bt Corn Event MON810

	FDA's "Note to the File"[54]	**Monsanto's Study[55]**
Nature of the inserted genetic material: **FDA: Complete copy of gene** **In fact: Partial gene**	"MON810 contains 1 ***complete copy*** of the *cryIA(b)* gene and its associated regulatory sequences." (p. 2)	"During the process of particle acceleration, ***the plasmid DNA can become broken resulting in the integration of partial genes*** into the genomic DNA. Southern blots and genomic clone sequence results described below established that the first 2448 bp of the 3468 bp *cryIA(b)* integrated into the corn line to produce MON810. In order to assess the protein products produced from the ***partial cryIA(b)*** gene…" (p. 14)
NOS 3' termination sequence: **FDA: NOS present** **In fact: NOS absent**	"***The NOS 3" nontranslated sequence served to terminate transcription*** of *cryIA(b)* [sic] gene, and to direct mRNA polyadenylation." (p. 2)	"…the *cryIA(b)* gene terminated its integration into the genomic DNA at position 2448 bp nucleotides of the *cryIA(b)* gene event. … The 2454 bp ***open reading frame*** codes for a protein containing amino acids 1-816 of the *B.t.k.* HD-1 protein[56] ***plus two additional amino acids [from corn] followed by a stop codon***." (p. 19; see also Figure 1)
Nature of the Bt protein: **FDA: nature-identical** **In fact: odd-length fragment**	"Monsanto states that the cryIA(b) protein present in MON809 and MON810is ***identical to that present in nature and commercial microbial preparations*** approved by the Environmental Protection Agency (EPA)." (p. 3)	"***The full length 131 kD B.t.k. HD-1 protein was not observed in line MON810***, as expected, since the full length gene was not incorporated into the corn genome. … ***The predicted molecular weight of the B.t.k. HD-1 protein from the partial cryIA(b) gene is 92 kD but is not detected***, probably due to low expression or rapid degradation to the trypsin-resistant product during the extraction procedure." (p. 15)

[54] FDA's Consultation Note for Monsanto's MON809 and MON810 Bt corn lines, September 18, 1996. See Memo for BNF No. 34 at http://www.cfsan.fda.gov/~lrd/biocon.html.

[55] Levine et al (1995). "Molecular Characterization of Insect-Protected Corn Line MON810," unpublished study submitted to the EPA by Monsanto, completed on May 30, 1995. EPA MRID No. 436655-01C.

[56] *B.t.k. = Bacillus thuringiensis*, subspecies *kurstaki.* HD-1 identifies the strain of *B.t.k.* from which the inserted gene was derived.