

HOLES IN THE BIOTECH SAFETY NET
FDA Policy Does Not Assure the Safety of Genetically Engineered Foods

by Doug Gurian-Sherman, Ph.D.

CENTER FOR SCIENCE IN THE PUBLIC INTEREST
1875 Connecticut Avenue, N.W., Suite 300
Washington, DC 20009-5728
Phone: (202) 332-9110
www.cspinet.org

TABLE OF CONTENTS

| SECTION | PAGE |
|---|------|
| EXECUTIVE SUMMARY | ii |
| I. INTRODUCTION..... | 1 |
| II. THE FDA REVIEW PROCESS..... | 1 |
| III. METHODS FOR CONDUCTING THIS REVIEW..... | 2 |
| IV. FINDINGS AND DISCUSSION..... | 4 |
| A. REQUESTS FOR ADDITIONAL DATA FROM FDA WERE IGNORED BY DEVELOPERS..... | 4 |
| B. FDA MISSED OBVIOUS ERRORS IN DATA SUMMARIES THAT A THOROUGH REVIEW WOULD HAVE UNCOVERED..... | 6 |
| C. INADEQUATE GUIDANCE ON SAFETY TESTS BY FDA RESULTS IN DATA SUMMARIES THAT LACK IMPORTANT SAFETY DATA | 7 |
| 1. <i>Toxicants and anti-nutrients that may affect food safety and nutrition are not always evaluated</i> | 7 |
| 2. <i>Inadequate methods used to determine allergenicity</i> | 9 |
| 3. <i>Inadequate determination of the toxicity of GE proteins</i> | 12 |
| 4. <i>Data summaries often lack sufficient detail or information to determine safety.</i> | 12 |
| 5. <i>Lack of guidance for determining that GE genes and proteins have not been altered in the transgenic plant</i> | 14 |
| a. <i>Alterations to the sequence of the transgene during transformation</i> | 14 |
| b. <i>Changes in the amino acid sequence of the transgenic protein due to transformation or alteration of the mRNA sequence during splicing</i> | 15 |
| c. <i>Post-translational modifications of the GE protein</i> | 15 |
| d. <i>Protein used in safety tests should be identical to the transgenic protein</i> | 16 |
| 6. <i>Lack of FDA guidance on safety testing in animals</i> | 16 |
| D. GENERAL CONCERNS ABOUT THE FDA REVIEW PROCESS..... | 17 |
| V. CONCLUSIONS AND RECOMMENDATIONS..... | 18 |
| VI. REFERENCES | 23 |

Doug Gurian-Sherman is Science Director for the Biotechnology Project at Center for Science in the Public Interest, a non-profit public interest organization based in Washington, D.C. He was formerly a scientist at the U.S. Environmental Protection Agency where he reviewed safety data for transgenic plants and microorganisms.

The author would like to acknowledge the assistance of Grace So and Rebecca Kane. In addition, several anonymous reviewers contributed many valuable suggestions. Finally, I would like to express my appreciation for the many contributions made by Greg Jaffe and Michael Jacobson, which greatly improved this report.

EXECUTIVE SUMMARY

The currently marketed genetically engineered (GE) crops, such as Bt corn and herbicide-tolerant soybeans, appear to be safe for consumers to eat. However, those few applications represent only a small fraction of the GE crops that could be on grocery store shelves in the future. This report assesses whether the Food and Drug Administration's (FDA) current oversight of GE crops is up to the task of ensuring the safety of those future biotech crops.

To determine the adequacy of FDA's current voluntary consultation process, we performed a detailed examination of more than a fourth of the data summaries (14 of 53) that FDA has reviewed. Our evaluation found that the biotechnology companies provide inadequate data to ensure their products are safe. In addition, it was clear from our review that FDA performs a less-than-thorough safety analysis. In particular, we found:

- **When FDA requested additional information to conduct a complete and thorough safety assessment, 50 percent (3 out of 6) of the time the GE-food developer did not comply with that request. In those cases, FDA had little choice but to complete its evaluation without the desired information.**
- **In three submissions, the data summaries contained obvious errors that were not identified by FDA during its review process.**
- **The submissions did not evaluate some potentially deleterious compounds, such as scientifically recognized toxicants in tomatoes or anti-nutrients in corn. In addition, allergenicity testing was not always performed using the best tests available.**
- **The data summaries reviewed by FDA often lacked sufficient detail, such as necessary statistical analyses needed for an adequate safety evaluation.**
- **FDA did not receive adequate data that the transgene and transgenic proteins were unaltered in the GE plant. Safety tests, such as for allergenicity, used forms of the protein that may differ from the GE protein found in the transgenic plant.**
- **FDA did not generate its own safety assessment, but merely summarized for the public the developer's food-safety analysis.**

Based on those findings, it is clear that FDA's current voluntary notification process (even if made mandatory) is not up to the task of ensuring the safety of future GE crops. To improve FDA's oversight of GE food crops and the public's confidence in GE foods, the FDA, or Congress where appropriate, should make the following changes:

- 1. Congress should provide FDA with legal authority for mandatory review and safety approval of GE crops, including the authority to require any data it deems necessary to conduct a thorough food-safety assessment.**

- 2. The FDA should develop detailed safety standards and testing guidelines.**
- 3. The FDA should require developers to submit not summaries of data, but complete details about their testing methods, the actual data from safety tests, and statistical analyses of those data.**
- 4. The FDA should establish an approval process which is transparent and provides the public with an opportunity to comment on submissions.**
- 5. The FDA should perform and make available to the public detailed assessments of commercialized GE crops.**
- 6. The FDA should reassess the safety of commercialized GE crops if new safety concerns are recognized or new tests become available.**

The enormous potential benefits from GE crops and foods will be fully realized only if FDA's regulatory system is significantly upgraded and enhanced. The changes we have recommended would cost little, but yield big dividends.

I. INTRODUCTION

The currently commercialized genetically engineered (GE) crops, such as Bt corn and herbicide tolerant soybeans and virus resistant papaya, appear to be safe to consumers. However, those few applications of GE technologies represent only a small fraction of GE crops that could be on grocery-store shelves in the future. Future GE food crops may include enhanced nutritional qualities and complex changes to a plant's metabolism, raising significant additional food-safety questions.

This report evaluates FDA's regulation of GE food and feed crops to determine whether FDA's voluntary consultation process can reliably assure food safety.¹ FDA is responsible for regulating the food safety of all GE crops with the exception of pesticidal genes and proteins, which are regulated by the U.S. Environmental Protection Agency (EPA). In contrast to FDA's voluntary consultation process for crops, pest-protected GE crops, GE animals, and food additives all undergo *mandatory* safety-approval by either EPA or FDA.²

This report focuses on answering such questions as: whether GE-crop developers supplied FDA with adequate data on the safety of the GE crop; whether developers complied with additional data requests by FDA; whether the supplied data summaries provided sufficient detail to allow adequate safety assessments; and whether FDA conducted a thorough review of the data summaries and provided a detailed discussion of the safety of the GE crop.

GE crops have the potential to provide substantial benefits to consumers, farmers, and the environment if properly developed. However, those benefits will only be realized if consumers, the ultimate arbiters in the acceptance of new technologies, have confidence in the safety of GE foods. That confidence will only be established if the FDA has a thorough and transparent regulatory process that assures the safety of each GE food.

II. THE FDA REVIEW PROCESS

FDA's policy for regulating biotech crops was set forth in 1992 (U. S. Food and Drug Administration, 1992), with subsequent guidance on consultation between GE-crop developers and FDA (U.S. Food and Drug Administration, 1997).³ The 1992 policy asserted that GE crops are usually the same as, or "substantially equivalent" to, the conventional non-GE crop. Therefore, like their conventional counterparts, they are considered "Generally Recognized as

1 FDA has responsibility for the safety of both food and animal feed. In this report, the term "food" may be broadly used to include both food and feed. In cases where the text considers only one or the other, it will be distinguished explicitly or by context.

2 EPA is responsible for food safety of pesticides under Section 408 of the Federal Food, Drug, and Cosmetic Act.

3 A recent proposed *mandatory* notification regulation (U.S. Food and Drug Administration, 2001) would require notification by the GE crop developer to the FDA of a proposed new GE crop and provide more transparency by giving the public access to the data summaries, but would not fundamentally change how safety reviews are conducted. It is not clear whether the FDA will finalize the proposed regulation.

Safe” (GRAS) under of the Federal Food, Drug, and Cosmetic Act (FFDCA) and no pre-market approval is necessary. However, FDA reserves the authority to apply the premarket approval requirements of FFDCA’s food-additive provisions to a GE crop if the crop may be or is shown to be harmful.

In its policy statement about biotech crops, FDA established a voluntary consultation process so it could review the developer’s determination of “substantial equivalence” before a biotech crop was marketed. Under the voluntary consultation process, the developer contacts FDA to discuss how it might establish substantial equivalence in a specific product. FDA provides guidance to help GE-food developers assess the safety of their GE crops. That guidance recommends that developers consider issues such as toxicity and allergenicity of the gene product and plant. “Decision trees” explaining general properties to consider in conducting the assessment are provided, but recommendations for testing and detailed testing procedures are not included.

After any informal discussions with FDA on conducting the food-safety assessment, the developer submits a document to FDA that summarizes the developer’s food-safety assessment, including summaries of data, tests performed, demonstration that the GE crop is essentially the same as the non-GE progenitor (“substantial equivalence”), and any interpretation and analysis of information it determines relevant to its assessment. FDA reviews that summary, meets with the developer if needed, and then an FDA staff person writes a memorandum called “Notes to File,” which constitutes the agency’s analysis. Finally, to complete the notification process, a letter is sent to the developer, saying that FDA has no further questions about the developer’s safety assessment and reminding the developer that it is its responsibility to assure that the food is safe. Between 1994 and 2001, FDA completed 53 voluntary consultations on biotech crops.

III. METHODS FOR CONDUCTING THIS REVIEW

To conduct this review, CSPI obtained all publicly available information from 14 of the 53 (26 percent) consultation packages (called Biotechnology Notification Files, or BNFs, by the FDA) using the Freedom of Information Act (FOIA) and FDA’s Center for Food Safety and Applied Nutrition (CFSAN) website. In most cases, the FDA files of several consultation packages were acquired for each engineered crop and transgene. For example, the consultations between FDA and several different developers of Bt corn were acquired. Similarly, several consultations were acquired for different crops, such as tomato and cantaloupe, that each contained the same transgene. Multiple consultations for the same crop species or the same gene allowed comparisons between consultations such as for thoroughness or inclusion of data. Consultations were also chosen to represent a range of genes and crop developers and to span the entire time period from the earliest to most recent consultations. The information obtained included: (1) the data summaries submitted by the developer; (2) communications between the developer and FDA; and (3) any analysis documents by FDA (Notes to File, letters to companies, etc.). Basic information about files examined for this study is found in Table 1 (see page 21).

For each submission, CSPI's review evaluated the following areas:

- 1) The adequacy of the developer's food-safety assessment and whether it complied with FDA's 1992 guidance and relevant scientific literature;
- 2) The testing and other information used to evaluate the toxicity and allergenicity of the GE protein;
- 3) The testing and other information used to ensure that the engineered plant did not express known toxicants and anti-nutrients at higher than normal levels;
- 4) The adequacy of the "substantial equivalence" analysis for both food and feed;
- 5) The completeness of the submissions and whether additional data were needed to satisfy FDA's concerns, and developers' responses to FDA requests for additional data; and
- 6) The thoroughness of FDA's review of the submission and its analysis of the safety of the product.

For this report, detailed analyses were usually restricted to the primary genes engineered into the crops, as well as the composition of the transgenic plant, rather than accessory genetic elements or marker genes. For example, the delayed-ripening gene of tomato and cantaloupe were examined but not antibiotic- or herbicide-resistance genes used for selection. Those other genes present many of the same issues as the reviewed genes. Several pesticidal genes that are under the jurisdiction of EPA are also not reviewed.⁴ In addition, specific parameters that should be evaluated, such as specific toxicants or anti-nutrients, have not been universally agreed upon. For that reason, we have taken a conservative approach concerning those parameters, basing our evaluation on criteria that we believe would be widely agreed upon.

Some issues of potential safety concern, such as whether the insertion of a transgene has disrupted a plant gene, are not considered. In other cases, issues that are considered in the report could have been explored more thoroughly. For example, changes in gene expression levels over time could have been addressed in addition to gene stability. Therefore, while extensive, this review is not exhaustive. However, we believe that sufficient examples have been considered to determine the adequacy of FDA's regulation of GE crops.

⁴ The primary added gene was under EPA jurisdiction in 7 of 14 submissions. For those 7 submissions, analysis was restricted to characteristics of the transgenic plant itself, which remain under FDA jurisdiction.

IV. FINDINGS AND DISCUSSION

A. REQUESTS FOR ADDITIONAL DATA FROM FDA WERE IGNORED BY DEVELOPERS

Six of the 14 FDA consultation files contained requests by FDA for additional information needed to fully assess food safety. In three (50 percent) of those cases FDA's requests were either ignored by the developer or the developer affirmatively declined to provide the requested information. FDA had to complete those reviews with less-than-thorough data summaries. FDA has no authority to require the developers to submit the desired additional data unless it decided to evaluate the crop as a food additive.

(i) In BNF-34, involving two Bt corn lines (MON 809 and MON 810), the data summary for MON 809 lacked composition data for the vegetative ("green", non-grain) part of the plant used as cattle forage. Compositional data is necessary to ensure the GE food is "substantially equivalent" to its non-GE counterpart, and that it is nutritious for livestock. Forage composition data was provided for MON 810, but those data do not mitigate the need for composition data about MON 809, since each transformation event is distinct and raises unique safety concerns. That is especially true where the transgenes are not the same. In this case, MON 809 contains an intact copy of a bacterial 5-enolpyruvalshikimate-3-phosphate synthase (CP4 EPSPS) gene as well as a Bt Cry1Ab gene, while MON 810 contains only the latter. Pleiotropic or other unexpected effects on forage equivalence that could occur due to the presence of CP4 EPSPS in MON 809 would not occur in MON 810.

In a telephone conversation with the developer, FDA "...inquired about the availability of additional information on forage composition for both MON 809 and MON 810..."⁵ The developer noted that no composition data were available for MON 809, whereupon FDA responded that it would be "unable to respond to inquiries about MON809 forage..." In its "Notes to File" FDA concludes that "Based on the information Monsanto has presented, we have no further questions about corn products containing the MON 810 transformation event or *grain* obtained from lines containing the MON 809 transformation event" (emphasis added). FDA included a similar statement in its concluding letter to the company for MON 809. In contrast, FDA concluded that it had no further questions concerning MON 809 grain because it found that adequate compositional and other data were provided for corn grain. Thus, unlike all other concluding letters reviewed for this report, FDA could not conclude that it had "no further questions" regarding MON 809 forage. But apparently FDA did not consider any questions to be large enough to trigger regulatory action.

(ii) In BNF-24 involving corn engineered with the Bt Cry1Ab protein, FDA determined that the data summary lacked composition analysis of the vegetative part of the plant. In a May

⁵ Based on an August 19, 1996 FDA memorandum.

12, 1995, telephone conference between the BNF-24 developer and FDA, FDA said that it would be “helpful” if the developer supplemented its submission with information about the nutritional value of the plant parts used as forage. In a reply dated May 30, 1995, the developer supplied only the protein and Cry1Ab content of the vegetative parts of the crop, as well as a discussion about agronomic properties as a surrogate for an adequate nutritional analysis. The developer did not provide any other nutritional composition data, but argued that if there were changes in the nutritional status of the plant, they would affect the agronomic properties as well. The developer gave no evidence of the relationship between agronomic properties, such as grain-yield and plant-growth characteristics, and nutritional composition.

In its analysis of BNF-24, FDA states that “CVM [Center for Veterinary Medicine] felt that the assessment would be strengthened by a proximate analysis of several nutritional components...” and “Alternatively, analysis of agronomic characteristics that correlate with nutritional components may suffice.”⁶ Neither the proximate analysis nor the correlation was provided by the developer. CVM concluded that it “...continues to believe that the assessment of the nutritional value of silage [a form of forage] derived from event 176 corn would be strengthened by analysis of other [than protein] nutritional components...of vegetative tissues.” Clearly FDA was not satisfied with the developer’s arguments and data, but could not require additional data.

(iii) In BNF-73 involving Bt Cry1F corn, FDA determined that the data summary had incomplete information about nutritional composition. FDA recommended to the developer that its “Composition data could be improved by addition of min/max [minimum and maximum] values to each analysis.” In a letter dated January 10, 2001, the developer responded to FDA’s request by stating “We believe the addition of minimum and maximum values for individual composition analysis will not significantly enhance the data set nor provide useful support for the safety conclusions in the current FDA notification” and suggested that it is more useful to compare average values from its GE variety to the “expected range” for the conventional crop. In other words, the developer decided to rely on its own judgment about what data were useful for risk assessment rather than FDA’s and did not comply with FDA’s suggestion.

The inability of FDA to require important data contrasts sharply with the safety-approval process for the registration of GE pesticidal crops at EPA, which often requires additional data. It may be argued that the stigma of ignoring FDA recommendations would cause GE-food developers to submit recommended data, but that was clearly not the case in several BNF submissions. It is worth noting that the data that FDA desired would not have been expensive or time consuming to obtain.

⁶ In its 1992 policy and guidance document, FDA notes that unexpected changes in deleterious compounds or nutrients may require specific test procedures, rather than a reliance on the status of agronomic traits such as tolerance to environmental stress or plant growth (U.S. Food and Drug Administration, 1992, p.22991).

B. FDA MISSED OBVIOUS ERRORS IN DATA SUMMARIES THAT A THOROUGH REVIEW WOULD HAVE UNCOVERED

We reviewed data summaries to determine if the developers' food-safety assessments are accurate and if FDA identified any problems with those assessments. In three of the 14 reviewed submissions, obvious errors were found that were not identified by FDA staff during their reviews of the submissions. Had FDA conducted thorough reviews, the errors would have been easily detected.

(i-ii) In BNF-14 and BNF-60, tomato and cantaloupe, respectively, were engineered with an *S*-adenosylmethionine hydrolase (SAMase) gene taken from bacterial virus T3. In both cases, the developer of those crops argued that natural exposure to SAMase in T3 found in the human digestive tract and drinking water supported a determination that the protein is safe for humans. (BNF-14, page 1 of the 1996 data summary and BNF-60, page 2, 1999 data summary). FDA considers previous dietary exposure to GE protein as important support for the safety of the GE crop.

While the submissions claim scientific support for prior dietary exposure to SAMase, the papers cited for that support (and included with the data summary) do not mention T3 or SAMase occurring in the gut (Furuse et al. 1993, Kott 1981) or in drinking water (Goyal et al., 1980). Another paper, included but not cited in the submission, states that "Coliphages [bacterial viruses] were not detected in finished drinking quality water" (Stetler, 1984).⁷ Therefore, contrary to the developer's conclusion, the cited papers do not support prior dietary exposure, and no other support for dietary exposure (such as detection of SAMase in the intestines) is provided.

There is no indication in publicly available files that FDA recognized the errors in the BNF-14 and BNF-60 data summaries. Reading of the developer-supplied and cited papers should have revealed the errors. Even without reviewing the reference papers, however, FDA should have questioned the developer's argument that the presence of T3 in gut bacteria that are found in the lower intestine (colon and lower small intestines (Savage, 1977)) is equivalent to dietary exposure where food passes through the entire digestive tract. Possible differences in the physiology between the upper and lower GI tracts invalidate the developer's argument.

(iii) In a third case, BNF-01 involving GE herbicide-tolerant soybeans containing the C4-EPSPS gene, the developer did not provide any nutritional composition data for forage and FDA did not address that omission. That contrasts with a later submission (BNF-55) that did address the composition of soybean forage. While soy forage is not a major part of a typical cattle diet, it

⁷ Although "finished water" is not defined, the Stetler (1984) paper indicates that he means water that has completed treatment at a drinking water facility.

remains a route of livestock exposure to GE soy and should have triggered an FDA data request.⁸ If FDA had conducted a thorough review, that omission of data would have been caught.

C. INADEQUATE GUIDANCE ON SAFETY TESTS BY FDA RESULTS IN DATA SUMMARIES THAT LACK IMPORTANT SAFETY DATA

FDA provides broad guidance to GE-food developers for determining the safety of GE crops, rather than suggesting specific types of safety tests or methods. We evaluated how GE-crop developers analyzed important safety parameters identified in the 1992 policy, including levels of known crop toxicants and anti-nutrients and the potential allergenicity and toxicity of GE proteins. Our review of 14 reviewed submissions found that developers do not evaluate all the compounds they should, and when they do, the methods they use are not always comparable to the contemporaneous state-of-the-art testing regimes.

1. Toxicants and anti-nutrients that may affect food safety and nutrition are not always evaluated

Many crops produce toxicants and anti-nutrients that may cause harm if expressed at higher levels in a GE crop than conventional counterparts. To address that potential safety concern, FDA's 1992 policy stated that the developer should "...assure that the new plant does not have significantly higher levels of toxicants than present in other edible varieties of the same species," but gave almost no guidance about which toxicants and anti-nutrients to measure. To determine if that safety concern was properly analyzed by GE-food developers, we reviewed which toxicants and anti-nutrients developers assessed in their submissions, compared different submissions that involved the same crop, and reviewed the scientific literature about known toxicants and anti-nutrients for those crops. We found that the inconsistent treatment of known toxicants and anti-nutrients by developers and FDA's lack of guidance on the subject resulted in submissions in which known toxicants and anti-nutrients were not analyzed before a GE crop was marketed.

(i-iii) Three GE-tomato submissions (BNF-02, BNF-14, and BNF-54) by three different developers⁹ were reviewed to compare assessments of toxicant alkaloid levels. Alkaloids are

⁸ BNF-55 cited EPA data that suggested that as much as 30% of cattle diet may consist of soy forage. The EPA data may overestimate the use of soy forage, since soybean experts estimate that less than 1% of soybean acres are now grown for that purpose, and USDA does not keep data on forage use (H. Ellison, USDA/NASS, S. Naeve, R. BreDahl, personal communication). Soy forage may also be used in emergency situations or when the soybean crop fails, for example due to drought (H. Ellison, USDA/NASS, S. Naeve, R. BreDahl, personal communication), and soybeans are also used as a component in a substantial amount of mixed grass plantings for forage (T.E. Devine, personal communication). Glyphosate application requirements for the use of glyphosate-resistant soybean forage are noted on current Roundup (glyphosate) labels. Since about 70 million acres of soybeans are currently harvested, even 1% of the crop is greater than the acreage of most other crops grown in the U.S. Therefore, even if forage is not a major use of the soy crop, a substantial amount may be used for cattle feed. Given the lack of accurate estimates of soybean forage acreage, inclusion of data on soy forage composition is prudent. If FDA considered the use of soy forage to be inconsequential, it did not say so. By contrast, FDA showed concern about possible feed issues with cantaloupe (BNF-60), a much smaller crop than soybeans and one that is not typically used for feed.

common and important toxicants in solanaceous plants, such as potato and tomato.¹⁰ In tomatoes, the alkaloid tomatine is predominant, but solanine and chaconine may also be found at low levels (Novak and Haslberger 2000). High levels of the alkaloid tomatine have been found in the ripe fruit of one non-GE tomato variant (Rick et al., 1994). All three alkaloids typically decrease during tomato ripening, and chaconine and solanine are often undetectable in ripe fruit.

In BNF- 02, where tomato was transformed with a 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD) gene, the developer measured levels of tomatine, chaconine, and solanine in green and ripe fruit. In BNF-14 and BNF-54, transformed with SAMase and Bt Cry1Ac respectively, only tomatine was measured. Neither the developers nor FDA discuss reasons for not providing data on chaconine or solanine. Since the possible unexpected increase of toxicants in GE crops is the primary reason for measuring them, the typically low levels of chaconine and solanine in tomato is not an adequate reason for not quantifying them. That is especially true because green tomato fruit, where concentrations may be higher, may be eaten.¹¹ Atypical plant gene expression, for example in the wrong tissue or developmental stage, has been widely observed due to naturally occurring mutations (e.g., Schneeberger et al., 1995, Marillonet and Wessler, 1997), and several cases of elevated expression involve toxicants (Rick et al., 1994, Zitnak and Johnston, 1970, Diawara and Trumble, 1997) in non-GE crops.¹² Thus, in two submissions, key tomato toxicants, which might have been altered by the genetic engineering of the crop, were not measured. While not all crop toxicants are currently known, those that have been identified should be measured. Doing so would not be expensive or time-consuming.

(iv-vi) We reviewed whether anti-nutrients were measured in four corn submissions (BNF-18, BNF-24, BNF-34, and BNF-73). Anti-nutrients are food components that inhibit the utilization of nutrients, so elevated levels may be harmful. Several anti-nutrients including phytate and trypsin inhibitor have been identified in corn (Novak and Haslberger, 2000). Phytate is widely recognized for reducing dietary availability of minerals, starch, and protein (Brinch-Pedersen et al. 2002, Manary et al. 2002, Novak and Haslberger, 2000). Phytate was measured in BNF-18 and BNF-73, but not BNF-24 or BNF-34. In addition, the level of trypsin inhibitor was reported in BNF-73 but not the other three corn submissions. The fact that both of those anti-nutrients were not measured in all engineered corn plants indicates that known corn food-safety concerns were not thoroughly assessed.

9 The BNF-54 data summary was submitted by Calgene. However, by the time of the BNF-54 consultation, Calgene was a subsidiary of Monsanto, which developed the BNF-02 tomato.

10 Like some chemical insecticides, they are inhibitors of cholinesterase. They also inhibit several other enzymes and may cause kidney inflammation, gastrointestinal problems, and interfere with iron absorption.

11 While the concentration of tomato alkaloids ordinarily decreases with ripening, GE changes in ripening could conceivably increase the alkaloid content in ripe fruit. Both SAMase and ACCase alter fruit ripening.

12 Novak and Haslberger (2000) also recommend measurement of oxalate and lectins, neither of which are assessed in any of the three tomato submissions.

Several previous reviews have expressed concern about the lack of detailed testing guidelines for toxicants and anti-nutrients. The National Academy of Sciences (NAS) recommended that FDA and EPA develop a database of toxicants and anti-nutrients (National Research Council, 2000) to address that problem. A previous European review of GE-food safety assessments found considerable inconsistencies in toxicants and anti-nutrients measured and recommended that to assure food safety “Consistent guidelines, specifying relevant compounds...have to be established” (Novak and Haslberger, 2000).

2. Inadequate methods used to determine allergenicity

We reviewed BNF files for allergenicity assessments, since allergens are an important and widely recognized possible risk in GE foods. The ability to introduce an allergen through GE was shown when a Brazil-nut protein introduced into soybean was identified by standard immunological assays to be a well-known allergen (Nordlee et al., 1996). The contamination of food corn with potentially allergenic StarLink corn, which was approved only for animal feed and other non-food use, reinforced the importance of determining the allergenicity of GE crops (Environmental Protection Agency, 2000 and 2001).¹³

In its 1992 policy, FDA provided no guidance for determining the allergenicity of proteins with no prior food use. FDA suggested that methods of sequence comparison might become more useful in the future. By 1996, several published guidance papers for assessing allergenicity were available for GE-food producers (Metcalf et al., 1996, Fuchs and Astwood, 1996). There are no definitive tests for allergenicity of a GE protein with no prior human exposure, but confidence is increased from the combined results of several available tests. Therefore, it is especially important that all applicable tests are used and properly conducted to give the best assessment possible. Important tests for allergenicity described in the 1996 papers include methods of sequence comparison between the GE protein and known allergens and testing the stability of the GE protein in a simulated gastric digestion (SGD) assay. Resistance to degradation in assays simulating the human stomach is a common property of food allergens and was the primary reason that the GE protein in StarLink corn was not approved for human consumption.

(i-ii) The importance of using proper methodology for SGD is illustrated in several studies where the relative ratios of the digestive enzyme (pepsin) to the food protein was varied to determine the effect on protein stability. In one case, a common plant protein that was rapidly digested at one ratio was stable when the pepsin concentration was reduced 100-fold (Astwood et al., 1996). Several recent studies also show that the stability of some known allergens can be reduced or eliminated when the relative pepsin concentration is increased (Fu, 2002 and Fu et al., 2002). In particular, some GE proteins that would be stable, and would be considered potential allergens, if tested according to Metcalfe et al. may be unstable at relatively high pepsin (or low GE protein) concentrations, and would pass the SGD assay. Therefore, using inappropriate concentrations of pepsin and the GE protein can potentially give misleading results.

¹³ The Starlink protein has not been shown to cause allergic reactions.

While pepsin and GE protein concentrations were not reported for SGD assays in any of the reviewed submissions, later papers on SGD written and published by the developer of BNF-01 and BNF-02 disclose the concentrations for pepsin, CP4 EPSPS, and ACCd (Harrison et al., 1996, Reed et al., 1996).¹⁴ Both BNFs report that CP4 EPSPS and ACCd were rapidly digested in SGD assays. The ratios of pepsin to CP4 EPSPS and ACCd were much higher in the SGD tests reported in the developer's papers (and presumably in the corresponding BNFs) than in several other papers published prior to BNF-01 and BNF-02, describing SGD tests for other proteins, or in more recent recommendations for GE allergen assessment.¹⁵ While the CP4 EPSPS and ACCd papers, and the BNFs, indicated that those proteins were unstable, it is not known whether that would have been true at higher CP4 EPSPS and ACCd or lower pepsin concentrations.

(iii) Of the reviewed submissions, only BNF-60 (for SAMase cantaloupe), from September 1998, was submitted after 1996 when allergenicity assessment methods became widely available and was otherwise suitable for review in this report.¹⁶ One important component of sequence comparison between known allergens and a new transgenic protein is to search for matches of at least eight consecutive amino acids, which may react with antibodies that induce an allergic response (Metcalf et al., 1996, Fuchs and Astwood, 1996). There was no mention in BNF-60 of homology assessment for the recommended eight consecutive amino acids.

Searching for overall sequence homology between the entire transgenic protein and known allergens is also recommended (Metcalf et al., 1996, Fuchs and Astwood, 1996). The BNF-60 submission stated only that the developer did not find any homology between SAMase and allergens, but provided no details about how that was determined other than noting the databases that were searched. As noted by Metcalf et al. (1996) and others (Gendel, 1998a, Gendel, 1998b), the methods used in searching for homology are important. Those methods were not disclosed in BNF-60.

14 BNF-01 and BNF-02 refer to internal developer documents for the digestibility studies, but do not supply them, or in the case of BNF-01, supplied the published paper several years after the FDA completed its review. Therefore it is not possible to say whether the published studies contain the same data as was referenced in the BNFs. However, the published studies remark that they were performed while following the FDA consultation process.

15 Calculations based on the data from those papers show that the ratios of pepsin to CP4 EPSPS and ACCd were 1650:1 and 800:1, respectively, while the ratios of pepsin to test protein used in several papers in the scientific literature (some published prior to BNF-01 or BNF-02) was about 100-fold to several thousand-fold less (Astwood et al., 1996, Fu, 2002, Table 1 and references therein, Fu et al., 2002). Recent international guidance on the use of SGD assay for GE proteins (United Nations Food and Agriculture Organization/World Health Organization, 2001) recommends a pepsin to test protein ratio of about 6.5:1, or about 120-fold and 250-fold proportionately less pepsin than was used in the tests for ACCd and CP4 EPSPS, respectively.

16 Other GE data summaries were not reviewed for allergenicity or toxicity, either because the GE protein is apparently not produced in the edible portion of the plant, or the GE pesticidal protein is under EPA jurisdiction.

Resistance to degradation in assays simulating the human stomach was the primary reason that the GE protein in StarLink corn was not approved for human consumption. Entire food-allergen proteins, or large fragments of them, remained undigested in SGD assays for at least two minutes (Metcalf et al., 1996, Fuchs and Astwood, 1996).¹⁷ In the BNF-60 SAMase SGD assay, the first measurement was not made until five minutes, which would not have detected resistance to digestion at between two and five minutes.

Antibodies are often used to detect digestion-resistant food-allergen proteins or fragments remaining after SGD, and several different types of antibodies that differ in their ability to detect the protein can be used. Polyclonal antibodies, which can typically detect several distinct sites, or epitopes, on a food-allergen protein are most appropriate because they may detect stable protein fragments that often result from the SGD tests. By contrast, monoclonal antibodies can detect only one type of epitope on the GE protein, and would not detect a digestion-resistant fragment that does not include that epitope. Monoclonal antibodies were used to detect SAMase during the SGD assays in BNF-60. Therefore, the detection method used in BNF-60 could have missed a digestion-resistant SAMase fragment.

SGD assays should be performed using the form of the protein found in the transgenic plant, because even minor alterations may change one or more of the properties of the protein. In tests reported in BNF-60, instead of testing SAMase itself, a SAMase fusion protein was used in digestive and heat-stability assays. A fusion protein is a single protein produced from a hybrid gene that joins two otherwise separate proteins. BNF-60, however, does not contain the fusion protein. It is possible that the fusion could have reduced the stability of the SAMase portion of the protein by altering its structure in the combined protein.¹⁸ FDA did not comment on any aspect of the allergenicity assessment of BNF-60.

In sum, four different problems were identified in the way the developer of SAMase performed or reported tests to show that SAMase is not likely to be allergenic. Together, such testing deficiencies denote an inadequate allergenicity analysis of SAMase.

Performing the assays described above in ways that would give more useful data would be no more costly than the methods used for the BNF tests.

17 In five of the seventeen known (non-GE) food allergens tested, a large fragment rather than the whole protein was the stable component.

18 It was noted in BNF-60 that the fusion protein, prior to digestion, retained SAMase activity indicating that the structure of the protein active site must be maintained. However, it remains possible that the junction between the two fused proteins is altered.

3. Inadequate determination of the toxicity of GE proteins

According to FDA (U.S. Food and Drug Administration, 1992), the potential toxicity of the introduced protein should be based on whether the biological functions of the protein raise concerns, whether the transgenic protein came from a non-GE food, and whether the transgenic protein is present in the GE-food in relatively large amounts (a macroconstituent). If the protein originated in a non-GE food source that occurs naturally in the diet, the FDA recommends determining post-translational differences and amount consumed of the GE and non-GE versions of the protein, and whether both food sources are processed similarly prior to eating (e.g. cooking). If those questions can be answered favorably, FDA does not have concerns about the toxicity of the GE protein. By contrast, in addition to the criteria used by FDA, EPA typically requires high-dose acute animal toxicity tests of GE pesticidal proteins under its jurisdiction.¹⁹

(i-ii) In BNF-14 and BNF-60, the developer compared the expected food exposure of GE-SAMase in the transgenic tomatoes and cantaloupes, respectively, to the estimated exposure that occurs for the non-GE source, the T3 bacterial virus.²⁰ To compare those exposures, the developer determined the estimated daily intake from T3/SAMase bacterial virus and the transgenic SAMase foods. In doing so, several questionable assumptions were made about the normal levels of T3-SAMase in human intestines and how much GE-SAMase would be consumed after commercialization. The developer assumes that 10 percent of intestinal *E. coli* are infected with T3, based on the 10 percent of *E. coli* reported to be susceptible to T3. However, the developer does not provide citations or data showing that T3 or SAMase is present at all in gut bacteria.

The developer asserts, without any substantiation, that SAMase does not have the properties of toxins. Its search of major sequence databases revealed no homology to toxins, but details were not given on how those searches were conducted other than listing the names of the databases. The FDA did not request additional data. Overall, the data summaries do not provide sufficient basis to assure lack of toxicity.

4. Data summaries often lack sufficient detail or information to determine safety.

FDA only reviews a summary of the data from the developer of the GE crop without defining how detailed the summary should be. How data are summarized and analyzed can have a large impact on the ability of FDA to determine if safety has been demonstrated, since highly

¹⁹ The difference between EPA and FDA guidance for toxicity tests is typically attributed to the different functions of GE proteins reviewed by the two agencies, since GE proteins reviewed at EPA often have known toxicity in contrast to proteins typically reviewed at FDA. However, we do not agree that FDA's disinterest in toxicity testing is always appropriate, especially if the source organism of the protein has no prior food use.

²⁰ As with our review of allergenicity assessment by FDA, GE proteins that are the jurisdiction of EPA, or are not expressed in the edible part of the GE crop were not reviewed.

summarized data lacking detail may not provide sufficient information to assure that proper data were collected and properly interpreted.

(i) Statistical analysis of numerical data is important for determining the significance of the data. For example, FDA considers gene stability over several generations of GE-crop growth to be an important part of the safety determination. A typical way to measure gene stability is to cross the GE plants with non-GE plants and determine whether the expected ratio of GE to non-GE phenotypes occurs in the progeny using the chi-square statistic. While all but three (BNF-43, BNF-48, and BNF-54) of the submissions provided transgene inheritance ratios as a measure of gene stability,²¹ seven of the submissions (BNF-01, BNF-02, BNF-14, BNF-24, BNF-32, BNF-45, and BNF-60) did not present chi-square (or any other) statistical analyses to verify the expected ratio of plants carrying the gene. By contrast, BNF-18, BNF-34, BNF-55, and BNF-73 did present chi-square analyses. FDA did not comment on the absence of statistical analyses in the BNFs we reviewed.

(ii) Another example of inadequate data summaries concerns test-crop growth conditions, which can have dramatic effects on crop composition values. For example, BNF-24 for GE corn supplied statistical analyses of crop nutritional composition compared to the non-GE progenitor, but no data on how the corn was grown. In contrast, BNF-18 gathered composition data about corn grown in six geographic locations and provided statistical analyses, but it pooled data from the different locations, which could obscure possible growth-condition effects. BNF-34, from the same company, did not disclose locations, pooled the composition data, and did not supply statistical analyses. GE-crop composition data is used to establish “substantial equivalence,” part of the determination that the crop is essentially the same as the non-GE variety and is the basis for avoiding evaluation as a food additive. In the absence of adequate data, the FDA cannot reliably determine substantial equivalence.

(iii) Inadequate detail for allergenicity assessments are found in BNF-01 and BNF-02, as noted in section IV.C.2 above, where the proportion of digestive enzyme (pepsin) to CP4 EPSPS and ACCd used to determine the digestive stability may have been inappropriately high, favoring digestion. Several papers examining food-protein digestibility that were published prior to BNF-01 and BNF-02 used pepsin-to-food-protein ratios thousands of times lower than in either FDA submission (see Fu, 2002, Table 1).

The basis for using the relative concentrations of pepsin to test proteins in BNF-01 and BNF-02 could not be evaluated by FDA because the methodology was not provided in the data summary. Had the relative concentrations of pepsin to CP4 EPSPS or ACCd been disclosed in BNF-01 or BNF-02, as would be the case in a full description of methodology, FDA may have had concerns about the allergenicity of the engineered proteins.

21 The three submissions without such analyses (BNF-43, BNF-48, and BNF-54), and one (BNF-24) without chi-square analyses, contain virus coat protein genes, coat protein and Bt genes, or Bt alone, so it is possible that additional data was submitted to EPA, which has jurisdiction over those genes.

All summarizing involves decisions about data to be disclosed or excluded, how to analyze that data for presentation, and how to interpret the results. Therefore, the more highly summarized and less detailed that data, the greater the role of the developer in determining the safety of the crop, and conversely the more the FDA must rely on the developer's judgment.

In several instances, which are noted above, safety-data summaries lack important information, such as statistical analysis. It is common for developers to present conclusions with little explanation of how experiments were conducted, leaving the FDA to accept the validity of the developers' conclusions or, alternatively, to request additional information. As shown in section IV.A. above, requests by the FDA for additional data are not typical and may even be rejected by the developer. Providing full details of experimental methods and results would add little if any expense for developers.

5. Lack of guidance for determining that GE genes and proteins have not been altered in the transgenic plant

FDA provides little guidance for assuring that potentially deleterious changes have not occurred in the transgene, and consequently to the GE protein, due to transformation of the plant. Those concerns include changes that may occur in the transgene or protein during transformation that alter the properties of the GE protein. While large numbers of transgenic plant tissue (calli) and plants are screened to make sure that they do not have obvious alterations, detection of many plant characteristics of health concern require specific testing. FDA should convene a group of independent outside experts, such as a science advisory panel or an NAS study committee, to help it determine how to assess possible changes in GE proteins.

a. Alterations to the sequence of the transgene during transformation

Rearrangement of the nucleotide sequence of a gene often occurs during the insertion of that gene into the genome of the recipient plant, especially when direct transfer methods such as microprojectile bombardment are used (Kohli et al., 1998, Pawlowski and Somers, 1998, Tinland, 1996). Most of those random changes impair or even eliminate the function of the protein coded by the gene and may be easily detected by bioassays. Some changes, however, may be more subtle and less easily determined. Even single nucleotide changes can alter a protein's amino acid sequence and affect the protein's properties.

It is important—and feasible—to determine the DNA sequence of the transgene (not just the protein sequence) because some regions of the transgene, such as the parts that control its expression, are not found in the protein. An altered sequence could indicate the value of looking more carefully at gene expression. However, none of the BNF submissions we reviewed, even

those submitted recently, determined those sequences.²² A recent report by the NAS recommended that transgene sequences should be provided to regulatory agencies unless justification for not doing so was provided (National Research Council, 2002).²³

b. Changes in the amino acid sequence of the transgenic protein due to transformation or alteration of the mRNA sequence during splicing

Some of the changes that might occur in the DNA sequence of the transgene during transformation will result in changes in the amino acid sequence of the transgenic protein. The structure of the engineered protein also may be changed by differences in mRNA splicing between the source of the gene and the recipient plant. Therefore, the amino acid sequence of the transgenic protein should be determined. The sequence can usually be determined from a small amount of protein, but none of the reviewed BNF submissions reported the sequences of the transgenic proteins.²⁴

c. Post-translational modifications of the GE protein

FDA recommends (U.S. Food and Drug Administration, 1992) that the GE-crop developer determine the presence of host-specific post-translational modifications of the GE protein, but gives no specific direction concerning the types of modification that should be considered or how to evaluate their significance. Post-translational modification involves chemical alterations, commonly by the addition of carbohydrate residues (termed glycosylation), after a protein is synthesized. Other post-translational changes, such as acetylation, phosphorylation, or methylation may also occur. Such alterations may change the structure, allergenicity, and other properties of a protein. Differences in post-translational modification between the gene-source organism and the recipient plant mean that safety evaluations based on the original protein may

22 The BNF files reviewed for this report begin in 1994, but even at that time, PCR and DNA sequencing were generally available and routinely used methods. In one case, BNF-18, partial DNA sequences of the plant-inserted DNA were reported, but not for the single intact copy of the Bt gene, Cry1Ab.

23 Most BNF submissions compare by sodium dodecyl sulfate-polyacrylamide gel electrophoresis the size of the transgenic protein with the original version produced in bacteria, but that process can reliably distinguish size differences only greater than about 5%-10%, and not differences of a few amino acids that may result from sequence changes occurring through transformation or splicing differences.

24 In higher organisms, the parts of mRNA coding for proteins (exons) are interspersed with non-coding sequences (introns) that are removed, or “spliced out,” prior to translation. The coding regions are then attached end-to-end and translated into the functional protein. Splicing patterns may be altered when a gene is placed in a new species, resulting in different forms of the protein. Those new proteins may have different properties than the protein produced in the original organism (Brown and Simpson, 1998). In plants, splicing changes caused by mutation or natural insertion of transpositional elements into introns of non-GE plants have caused changes in gene expression (Marillonnet, 1997, Sablowski, 1998), altering the growth of the plant. Splicing changes can be found by several methods, such as by determining the sequence of DNA after conversion *in vitro* from spliced mRNA, but that has not been reported in any of the BNF submissions.

not be reliable. The exact post-translational structure(s) of the protein as synthesized in the GE plant should be determined and the properties and effects of that protein should be part of allergenicity assessments and toxicity tests. Of the BNF files that we reviewed for testing of post-translational modification, the only modification that was considered was whether the transgenic protein was glycosylated.²⁵ Developers did not examine any other possible post-translational modifications.

d. Protein used in safety tests should be identical to the transgenic protein

Allergenicity and toxicity tests may not provide useful results if they use proteins produced in organisms other than the transgenic plants. That is because those proteins may have been modified differently (or not modified at all) by DNA sequence changes during transformation, mRNA changes, or post-translational alterations. Due to the difficulty of obtaining sufficient purified transgenic protein from the GE plant, developers conduct such tests using protein obtained from bacteria containing the engineered gene, which can produce more of the protein.²⁶ However, bacterial proteins are not glycosylated and their genes are never spliced, whereas proteins in plants and other higher organisms often are. Therefore, bacterially produced protein may not be identical to, and have the same health effects as, the GE protein from the plant. Limited FDA guidance concerning such potential differences can result in unfounded safety conclusions. Therefore, proteins produced in organisms other than the GE plant should only be used in safety tests and as the basis of safety decisions if they have been shown to be identical to the transgenic-plant form of the protein.

6. *Lack of FDA guidance on safety testing in animals*

FDA provides no guidance on whether and how to conduct animal-feeding toxicity studies. Such studies are commonly conducted for pesticides (including plant-incorporated protectants) and food additives. Animal-feeding studies using GE plants are one way of partially addressing potential health effects of the GE proteins, as well as unintentional effects of transformation on GE plants. The value of whole-plant testing is controversial, because it is not possible to expose the animals to a high dose of the engineered plant, which is needed to provide adequate sensitivity. In addition, in some cases test animals may be less sensitive than humans to deleterious effects, in which case adverse effects may only be detected at higher doses. To compensate for the lack of a high dose, impractically large numbers of animals would need to be used. Feeding tests also may yield false-positive findings, just due to chance, and in some cases animals may have adverse reactions that would not occur in humans. Both of those possibilities could lead to costly and unnecessary further testing.

²⁵ As with other issues, transgenes that were not the responsibility of EPA and expressed the transgenic protein in the edible portion of the plant were evaluated.

²⁶ Other microorganisms, especially yeasts, may be used to produce large amounts of protein, but have not been used to produce proteins for the reviewed BNFs.

On the other hand, conducting feeding studies should be considered because they might detect problems and they would add public confidence to safety determinations of a new technology with less-than-perfect testing protocols for assessing food safety. For example, unexpected effects are currently addressed primarily by targeted screening for changes in a limited number of plant characteristics, such as known toxicants, nutrients, and anti-nutrients. In contrast, animal feeding studies may detect problems caused by changes other than those previously known to cause safety problems. Similarly, possible effects of any changes in the GE protein in the plant could be assessed in tests of the isolated protein. Of the 14 BNF submissions reviewed, six conducted whole-GE-plant animal-feeding studies (BNF-01, BNF-02, BNF-18, BNF-24, BNF-32, and BNF-55). Those studies varied widely in rigor and detail. A recent NAS report recommended further study of the utility of animal feeding studies (National Research Council, 2000), and a current NAS committee²⁷ may consider this issue. The issue of whether and how animal-feeding toxicology studies should be conducted should be evaluated by the NAS. FDA should carefully consider any advice proffered by the current NAS committee or solicit additional expert advice on this issue if that committee does not fully address it.

D. GENERAL CONCERNS ABOUT THE FDA REVIEW PROCESS

There are several concerns about the FDA review process due to the primary role that the GE crop developer plays in determining the safety of the GE crop. One result of that reliance of FDA on the judgment of the food developer is reflected in the perfunctory safety analysis expressed in FDA's review documents, called "Notes to File." Those documents typically only reiterate the developers' assessments and contain statements attributing safety conclusions to the GE developer rather than to FDA. The letter concluding the consultation between FDA and the developer clearly places responsibility for the safety of the GE food with the developer. In that letter, FDA tells the developer that "...it is our understanding that [the developer] has concluded that...[the GE food] does not raise issues that would require pre-market review or approval of FDA." Indeed, the lack of thorough data in BNFs may preclude the FDA from conducting adequate reviews and could result in unsafe GE foods entering the marketplace.

Since FDA does not define what it means by "data summary," the amount of detail submitted to the agency varies greatly among BNF data packages. Some submissions are hundreds of pages long while others are only 10 or 20. The latitude given to GE-food developers by FDA contrasts with safety-approval processes, such as for food additives or pesticides, where detailed safety-testing guidelines are often specified and certain data are required by the FDA or EPA. The need for detailed, though flexible, testing guidelines stems from the recognition that performing the wrong tests, not performing needed tests, or carrying out the tests using

27 The committee is under the auspices of the of NAS Board on Life Sciences, Food and Nutrition Board, and the Board on Agriculture and Natural Resources, and is titled "Process to Identify Hazards and Assess the Unintended Effects of Genetically Engineered Foods on Human Health."

inappropriate methods can give misleading results, miss important safety concerns, waste the government's and company's time and money, and endanger consumers.

V. CONCLUSIONS AND RECOMMENDATIONS

The FDA consultation process does not allow the agency to require submission of data, misses obvious errors in company-submitted data summaries, provides insufficient testing guidance, and does not require sufficiently detailed data to enable the FDA to assure that GE crops are safe to eat. Under the current process, FDA largely appears to rely on the developer's judgment about what data it should provide to the FDA. That results in a conflict between the developer's need to market the GE crop and the public's need for assurance of safety. The Agency's proposed mandatory notification process²⁸ would add transparency and assure that all GE-food crops are reviewed by FDA, but would not fundamentally change the way they are evaluated.

Those inadequacies will be exacerbated in the future when more complex changes are made in the metabolism of plants and a wider variety of genes are utilized. Current GE foods involve only simple genetic changes, typically introducing only one or two new proteins that are not intended to change the plant's metabolism. Despite increasing knowledge about metabolic pathways in plants, genetic engineering can cause unexpected or pleiotropic effects (Atkinson et al., 2002, Bergelson et al., 1996, Conner and Jacobs, 1999, Gutierrez-Campos et al., 2001, Osusky et al., 2000, Sharkey et al., 1991), including a recent example of unexpectedly increased lycopene in non-commercialized tomatoes engineered at the U.S. Department of Agriculture and several universities for delayed ripening (Mehta et al., 2002). While increased lycopene (a carotenoid) levels might be beneficial to human health, that unexpected change illustrates the unpredictability of metabolic engineering. The current voluntary consultation process for testing GE crops will not address those more complex changes. Improvements need to be made while riskier applications of biotechnology are still in their infancy and before safety problems occur.

The problems discussed in this report demonstrate that the FDA's current safety-review process needs to be strengthened. To improve the quality of FDA's regulatory oversight of GE-food crops and to improve public confidence in the safety of foods made from those crops, the FDA should make the following changes. Where the FDA lacks the authority to implement some of these recommendations, Congress should pass new legislation.

- 1. Congress should provide FDA with legal authority for mandatory review and safety approval of GE crops, including the authority to require any data it deems necessary to conduct a thorough food-safety assessment.** Based on our review, in three of six cases where FDA believed it needed additional data to assess a GE food's safety, the developer did not provide those data. To thoroughly assess the safety of a GE crop, FDA needs the authority to

28 As noted in footnote 3.

require necessary information. Currently, FDA does not have that authority. A mandatory approval process also would give the public in the U.S. and abroad greater confidence that GE foods were safe. If FDA were required to affirm the safety of GE foods, it would be more likely to conduct a rigorous safety analysis.

2. The FDA should develop detailed safety standards and testing guidelines.

The current lack of detailed guidance on tests to be performed results in submissions that do not include all the data needed to conduct a safety evaluation. FDA needs to develop testing standards and guidelines for GE crops and revise those guidelines as the science evolves. Without detailed guidance explaining what tests to perform and the conditions under which to perform them, FDA will be unable to ensure that GE crops are safe to eat.

3. The FDA should require developers to submit not summaries of data, but complete detail about their testing methods, the actual data from safety tests, and statistical analyses of those data.

The acceptance of data summaries rather than actual data prevents FDA from reviewing important safety information. FDA needs to see all of the data that developers produce, including the details concerning conditions under which tests are performed, the number of generations tested, the locations of test plots, and numerous other pieces of scientific information critical to assessing the crop's safety.

4. The FDA should establish an approval process which is transparent and provides the public with an opportunity to comment on submissions.

Some of the problems identified in the developers' submissions and FDA's reviews of those submissions (factual mistakes, incomplete analysis of toxicants, inadequate testing for allergenicity, etc.) might be avoided if the public had easy access to, and an opportunity to provide FDA with comments on, those submissions. Experts outside the FDA might assist the agency by identifying problems or errors. At the very least, public scrutiny would encourage FDA to conduct the most careful and thorough reviews.

5. The FDA should perform and make available to the public detailed assessments of commercialized GE crops.

FDA's current analyses of the safety of GE foods do not truly analyze the data summaries provided by the developers. FDA merely restates and summarizes the developers' food-safety analyses. If FDA provided the public via the Federal Register and its own internet site with its own detailed analyses of the safety of GE crops, it would review submissions more carefully, catch obvious mistakes and omissions, and confirm or question the safety of foods made from them. Such an explanation should increase the public's confidence in FDA's decisions.

6. The FDA should reassess the safety of commercialized GE crops if new safety concerns are recognized or new tests become available.

Available tests for several safety concerns are inadequate. For example, currently no tests can definitively predict allergenicity or assure that unexpected adverse changes, such as increased levels of substances not currently recognized as deleterious, have not occurred in the plant. When such tests become available (such as an animal model predictive of human allergens), the FDA should require developers to provide data on previously commercialized crops within a reasonable period of time.

7. The FDA should ask developers of current GE crops to provide additional data to give greater assurance of safety than the summary data previously given to the agency.

Although available data (the FDA and EPA submissions, as well as other publicly available studies of the food safety of individual products) on currently commercialized crops suggest that those crops are safe to eat, a complete and thorough food-safety data package could further ensure the safety of and public confidence in commercialized GE crops. Thus, FDA should obtain from the developers and release to the public all underlying data supporting their products. It would cost developers little to provide both the data supporting their submissions to FDA and any subsequent testing that was performed. In addition, to the extent that past submissions did not include all testing for what now would be considered a thorough safety assessment (such as not conducting sufficient testing for toxicants), FDA should request that those tests be performed. Because many of the missing data could be obtained from *in vitro* tests, the cost of providing the new data would be minor.

Scientific bodies such as the NAS and others have stated that the risks from GE foods can be addressed by adequate regulation. Unfortunately, the FDA's current notification process is not up to the task. The enormous potential benefits from GE crops and foods will be fully realized only if the FDA's regulatory system is upgraded to assure consumer safety. The changes we have recommended would cost little, but would yield big dividends.

Table 1: Biotechnology Notifications Reviewed for This Report

| Host Crop Species | FDA BNF-# | Primary Transgenic Proteins | GE Traits | Source Organism | Developer | Date of Submission/Date of Completion |
|--|---------------|---|---------------------------------|--|---|--|
| Soybeans (<i>Glycine max</i>) | BNF-01 | CP4-EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) | Glyphosate herbicide resistance | <i>Agrobacterium</i> sp. strain CP4 | Monsanto Co. | Submitted: January 20, 1994 Completed: January 27, 1995 |
| Tomato (<i>Lycopersicon esculentum</i>) | BNF-02 | ACC d (1-aminopropane-1-carboxylic acid deaminase) | Altered fruit ripening | <i>Pseudomonas chlororaphis</i> | Monsanto Co. | Submitted: August 23, 1994 Completed: April 5, 1995 |
| Tomato (<i>Lycopersicon esculentum</i> var. <i>cerasiforme</i>) | BNF-14 | SAMase (S-adenosylmethionine hydrolase) | Altered fruit ripening | <i>Escherichia coli</i> bacteriophage T3 | Exelixis Plant Sciences Inc. (formerly Agritope Inc.) | Submitted: September 21, 1994 Completed: March 20, 1996 |
| Corn (<i>Zea mays</i>) | BNF-18 | Cry1Ab | Insect resistance | <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> | Monsanto Co. | Submitted: September 15, 1995 Completed: July 24, 1996 |
| Corn (<i>Zea mays</i>) | BNF-24 | Cry1Ab | Insect resistance | <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> | CIBA-Geigy (now Syngenta) | Submitted: January 14, 1993 Completed: July 14, 1995 |
| Oilseed Rape (<i>Brassica napus</i>) | BNF-32 | Barnase and BARstar | Male plant sterility | <i>Bacillus amyloliquefaciens</i> | Plant Genetics Systems (part of Aventis) | Submitted: October 25, 1995 Completed: April 4, 1996 |
| Corn (<i>Zea mays</i>) | BNF-34 | Cry1Ab | Insect resistance | <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> | Monsanto Co. | Submitted: June 6, 1996 Completed: September 25, 1996 |
| Yellow Crookneck Squash (<i>Cucurbita pepo</i> sp. <i>ovifera</i>) | BNF-43 | CMV, ZYMV, WMV2 coat proteins | Virus resistance | Cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV), and watermelon mosaic virus 2 (WMV2) | Seminis Vegetable Seeds | Submitted: March 3, 1997 Completed: July 10, 1997 |
| Radicchio (<i>Chicorium intybus</i>) | BNF-45 | Barnase | Male plant sterility | <i>Bacillus amyloliquefaciens</i> | Bejo Zaden BV, Holland | Submitted: May 20, 1997 Completed: October 22, 1997 |

| Host Crop Species | FDA BNF-# | Primary Transgenic Proteins | GE Traits | Source Organism | Developer | Date of Submission/Date of Completion |
|---|------------------|---|----------------------------------|---|---|--|
| Potato (<i>Solanum tuberosum</i>) | BNF-48 | Cry3A, PLRV replicase | Insect and virus resistance | <i>Bacillus thuringiensis</i> subsp. <i>tenebrionis</i> (Btt); Potato leafroll virus (PLRV) | Monsanto Co. | Submitted: July 21, 1997 Completed: January 8, 1998 |
| Tomato (<i>L. esculentum</i>) | BNF-54 | Bt Cry1Ac | Insect resistance | <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> (Btk) | Calgene (Division of Monsanto Co.) | Submitted: December 22, 1997 Completed: February 24, 1998 |
| Soybeans (<i>Glycine max</i>) | BNF-55 | PAT Phosphothricine acyl transferase | Glufosinate herbicide resistance | <i>Streptomyces viridochromogenes</i> | AgrEvo (now Aventis) | Submitted: March 21, 1998 Completed: May 15, 1998 |
| Cantaloupe (<i>Cucumis melo</i>) | BNF-60 | SAMase (S-adenosylmethionine hydrolase) | Altered fruit ripening | <i>E. coli</i> bacteriophage T3 | Exelixis Plant Sciences Inc. (formerly Agritope Inc.) | Submitted: May 5, 1999 Completed: December 9, 1999 |
| Corn (<i>Zea mays</i>) | BNF-73 | Cry1F | Insect resistance | <i>Bacillus thuringiensis</i> | Dow AgroSciences | Submitted: June 28, 2000 Completed: May 18, 2001 |

VI. REFERENCES

- Astwood JD, Leach JN, and Fuch RL (1996) Stability of food allergens to digestion in vitro. *Nature Biotech.* **14**:1269-1273
- Atkinson R.G., Schröder, R., Hallett, I.C., Cohen D., and MacRae, E.A. (2002) Overexpression of polygalacturonase in transgenic apple trees leads to a range of novel phenotypes involving changes in cell adhesion. *Nature Biotech.* **129**:122-133
- Bergelson J., Purrington, C.B., Palm, C.J., and López-Gutiérrez, J. (1996) Costs of Resistance: a test using transgenic *Arabidopsis thaliana*. *Proc. R. Lond. B Biol. Sci.* **263**(1377): 1659-1663
- BreDahl, R. (2002) Iowa State University Extension, personal telephone communication. July 29, 2002
- Brinch-Pedersen H., Sorensen, L.D. and Holm, P.B. (2002) Engineering crop plants: getting a handle on phosphate. *Trends Plant Sci.* **7**(3):118-125
- Brown, J.W.S. and Simpson, C.G. (1998) Splice site selection in plant pre-mRNA splicing. *Ann. Rev. Plant. Physiol. Plant Mol. Biol.* **49**:77-95
- Conner, A.J. and Jacobs, J.M. (1999) Genetic engineering of crop as potential source of genetic hazard in the human diet. *Mutat. Res.* **443**(1-2): 223-234
- Devine, T.E. (2002) U.S. Department of Agriculture, personal telephone communication. July 29, 2002
- Diawara, M.M. and Trumble, J.T. (1997) Linear furanocumarins. "Handbook of Plant and Fungal Toxicants," D'Mello J.P. ed. CRC Press, Boca Raton, FL
- Ellison, H. (2002) U.S. Department of Agriculture/National Agricultural Statistics Service, personal telephone communication. July 23, 2002
- Fu, T-J (2002) Digestion stability as a criterion for protein allergenicity assessment. *Ann. N.Y. Acad. Sci.* **964**:99-110
- Fu, T-J, Abbott, UR, Hatzos, C. (2002) Digestibility of food allergens and nonallergenic proteins in simulated gastric fluid and simulated intestinal fluid – a comparative study. *J. Agric. Food Chem.* **50**:7154-7160
- Fuchs, R.L. and Astwood, J.D. (1996) Allergenicity assessment of foods derived from genetically modified plants. *Food Technol.* **50**(2):83-88

Furuse, K., Osawa, S., Kawashiro, J., Tanaka, R., Ozawa, A., Sawamura, S., Yanagawa, Y., Nagao, T., and Watanabe, I. (1983) Bacteriophage distribution in human feces: continuous survey of healthy subjects and patients with internal and leukaemic diseases. *J. Gen. Virol.* **64** (Pt 9):2039-43

Gendel, S.M. (1998a) The use of amino acid sequence alignments to assess potential allergenicity of proteins used in genetically modified foods. *Adv. Food Nutr. Res.* **42**:45-62

Gendel, S.M. (1998b) Sequence databases for assessing the potential allergenicity of proteins used in transgenic foods. *Adv. Food Nutr. Res.* **42**:63-92

Goyal, S.M., Zerda, K.S., and Gerba, C.P. (1980) Concentration of coliphages from large volumes of water and wastewater. *Appl. Environ. Microbiol.* **39**(1):85-91

Gutierrez-Campos, R., Torres-Acosta, J.A., Pérez-Martínez, J.J., and Gómez-Lim, M.A. (2001) Pleiotropic effects in transgenic tobacco plants expressing the oryzacystatin I gene. *HortSci.* **36**(1): 18-19

Harrison, L.A., Bailey, M.R., Naylor, M.W., Ream, J.E., Hammond, B.G., Nida, D.L., Burnette, B.L., Nickson, T.E., Mitsky, T.A., Taylor, M.L., Fuchs, R.L., and Padgett, S.R. (1996) The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. *J. Nutrition* **126**(3):728-740

Kohli, A., Leech, M., Vain, P., Laurie, D.A., and Christou, P. (1998) Transgene organization in rice engineered through direct DNA transfer supports a two-phase integration mechanism mediated by the establishment of integration hot spots. *Proc. Natl. Acad. Sci. USA* **95**: 7203-7208

Kott, Y. (1981) Viruses and bacteriophages. *Sci. Tot. Environ.* **18**:13-23

Manary M.J., Hotz, C., Krebs, N.F., Gibson, R.S., Westcott, J.E., Broadhead, R.L., and Hambidge, K.M. (2002) Zinc homeostasis in Malawian children consuming a high-phytate, maize-based diet. *Am. J. Clin. Nutr.* **75**(6):1057-1061

Marillonet, S., and Wessler, S.R. (1997) Retrotransposon insertion into the maize *waxy* gene results in tissue-specific RNA processing. *Plant Cell* **9**:967-978

Mehta, R.A., Cassol, T., Li, N., Ali, N., Handa, A.K., and Matoo, A.K. (2002) Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality, and vine life. *Nature Biotechnol.* **20**(6):613-618

Metcalf, D.D., Fuchs, R.L., Townsend, R., Sampson, H.A., Taylor, S.L., and Fordham, J.R. (1996) Assessment of the allergenic potential of foods derived from genetically engineered crop plants. *Crit. Rev. Food Sci. Nutr.* **36**(S):S165-S186

Naeve, S. (2002) Minnesota Extension Service, personal telephone communication. July 25, 2002

National Research Council. (2000) "Genetically Modified Pest-Protected Plants: Science and Regulation." National Academy Press, Washington, D.C.

National Research Council. (2002) "Environmental Effects of Transgenic Plants: The Scope and Adequacy of Regulation." National Academy Press, Washington, D.C.

Nordlee, J.A., Taylor, S.L., Townsend, J.A., Thomas, L.A., and Bush, R.K. (1996) Identification of a Brazil-nut allergen in transgenic soybeans. *New Eng. J. Med.* **334**(11):688-692

Novak, W.K., and Haslberger, A.G. (2000) Substantial equivalence of antinutrients and inherent plant toxicants in genetically modified foods. *Food Chem. Toxicol.* **38**:473-483

Osusky, M., Zhou, G., Osuska, L., Hancock, R.E., Kay, W.W., and Misra, S. (2000) Transgenic plants expressing cationic peptide chimeras exhibit broad-spectrum resistance to phytopathogens. *Nature Biotech.* **18**: 1162-1166

Pawlowski, W.P. and Somers, D.A. (1998) Transgenic DNA integrated into the oat genome is frequently interspersed by host DNA. *Proc. Natl. Acad. Sci. USA* **95**: 12106-12110

Reed, A.J., Kretzmer, K.A., Naylor, M.W., *et al.* (1996) Safety assessment of 1-aminocyclopropane-1-carboxylic acid deaminase protein expressed in delayed ripening tomatoes. *J. Agric. Food Chem.* **44**:388-394

Rick, C.M., Uhlig, J.W., and Jones, A.D. (1994) High α -tomatine content in ripe fruit of Andean *Lycopersicon esculentum* var. *cerasiforme*: developmental and genetic aspects. *Proc. Natl. Acad. Sci. USA* **91**:12877-12881

Sablowski, R.W.M. and Meyerowitz, E.M. (1998) Temperature-sensitive splicing in the floral homeotic mutant *apetala3-1*. *Plant Cell* **10**:1453-1463

Savage, D.C. (1977) Microbial ecology of the gastrointestinal tract. *Ann Rev Microbiol.* **31**:107-133

Schneeberger R.G., Becraft, P.W., Hake, S., Freeling, M. (1995) Ectopic expression of the *knox* homeo box gene *rough sheath1* alters cell fate in the maize leaf. *Genes and Devel.* **9**:2292-2304

- Sharkey T.D., Vassey, T.L., Vanderveer, P.J., and Vierstra, R.D. (1991) Carbon metabolism and photosynthesis in transgenic tobacco (*Nicotiana tabacum* L.) having excess phytochrome. *Planta* **185**: 287-296
- Stetler, R.E. (1984) Coliphages as indicators of enteroviruses. *Appl. Environ. Microbiol.* **48**(3): 668-670
- Tinland, B. (1996) The integration of T-DNA into plant genomes. *Trends Plant Sci.* **1**(6): 178-184
- United Nations Food and Agriculture Organization/World Health Organization. (2001) "Evaluation of Allergenicity of Genetically Modified Foods, Food and Agriculture Organization." Rome, Italy. January 22-25, 2001
- U. S. Environmental Protection Agency. (2000) SAP report No. 2000-06: "Assessment of Scientific Information Concerning StarLink™ Corn." www.epa.gov/scipoly/sap/2000/index.htm
- U. S. Environmental Protection Agency. (2000) SAP report No. 2001-09: "Assessment of Additional Scientific Information Concerning StarLink™ Corn." www.epa.gov/scipoly/sap/2001/index.htm
- U.S. Food and Drug Administration, (1992), Federal Register "Statement of Policy: Foods Derived from New Plant Varieties." Vol. 57 No. 104, p.22984-22992. May 29, 1992
- U.S. Food and Drug Administration, (2001), Federal Register "Premarket Notice Concerning Bioengineered Foods." Vol. 66, p.4706-4738, January 18, 2001
- U.S. Food and Drug Administration. (1994) Center for Food Safety and Applied Nutrition. "Conference on Scientific Issues Related to Potential Allergenicity in Transgenic Food Crops." <http://www.cfsan.fda.gov/~lrd/bioallrg.html>
- U.S. Food and Drug Administration. (1997) Center for Food Safety and Applied Nutrition. "Guidance on Consultation Procedures Foods Derived From New Plant Varieties." October 1997. <http://www.cfsan.fda.gov/~lrd/consulpr.html>
- Zitnak, A., and Johnston, G.R. (1970) Glycoalkaloid content of B5141-6 potatoes. *Am. Potato J.* **47**:256-260