Biohazards: The Next Generation?

Crop Plants that Manufacture Industrial and Pharmaceutical Proteins

a draft preliminary report from The Edmonds Institute

Concern:

The Starlink corn scandal has already demonstrated that a genetically engineered corn variety not approved for human consumption could gain access to the international food supply despite government regulation and industry oversight. Unfortunately, Starlink may not be the only problem. Other plants that look like food - those being developed and grown to manufacture industrial and pharmaceutical proteins - may hold even greater hazard for the unsuspecting consumer than the potentially allergenic Starlink corn.

Emerging questions:

How can countries ensure that genetically engineered crops grown to produce a variety of industrial chemicals and pharmaceuticals do not contaminate food and seed supplies? If companies cannot contain problematic genes and crops, should they be growing them? If companies insist on growing them, should they be required to ensure that genes not generally approved for human consumption be accompanied by a marker that makes their presence visually apparent -- in order that products containing those genes cannot pass unnoticed into other plants, into the food supply, or across the border?

Related Questions - aside from questions of liability - for the Intergovernmental Committee for the Cartagena Protocol on Biosafety:

How can personnel at the border validate that a shipment is/contains only what its identifying documents say it is/contains **and nothing more**?

What information in the accompanying documents will help border personnel check that the product is/contains the organism as described **and nothing more**? What training/ support/ certification will border personnel require to help them utilize this information properly?

Will sufficient information be made available to the biosafety clearing house in advance of shipment so that designated personnel may be able to answer questions of product/organism composition at the border?

Will sufficient information - in electronic form (e.g., diskettes and CDs) and in printed form - accompany the shipment so that designated personnel may be able to answer questions of product/organism composition at the border?

Who is to pay the cost of testing to ensure that the accompanying documents are fully accurate?

What procedures are to be followed:
-- if the accompanying documents are found to be inaccurate at the border?
-- if the accompanying documents are found to be inaccurate after the shipment has passed the border?

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compiled by

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for

The Edmonds Institute

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Note: This report is a work in progress. We apologize for printing it without all the necessary citations. A revised version will be forthcoming. Readers are invited to share their comments or criticisms with:

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Introduction

The global controversy over genetically engineered foods has spurred a crisis of confidence for the biotechnology industry and its investors. Despite the expenditure of $50 million this year to promote the benefits of biotechnology in the U.S., the recent discovery there of products contaminated with a variety of Aventis Bt corn that is not approved for human consumption has once again put the industry on the defensive.

The prevailing corporate strategy of the late 1990s—when a hoped-for synergy between agricultural and pharmaceutical biotechnology led to the development of a few giant "life science" conglomerates—has now been badly shaken. In the last year, Monsanto's agricultural division has been spun off from its recent parent company Pharmacia, Syngenta, a separate agribusiness company, has been created from the relevant divisions of Zeneca and Novartis, and Aventis has announced it will seek to separate out its agbiotech divisions.

Nevertheless, significant technological and financial synergies remain between agricultural and pharmaceutical biotechnology. Further, the efforts of the biotechnology industry to portray itself as a humanistic force in the world rest significantly on blurring the distinctions between biotechnology for food and biotechnology for medicine. The industry's strategy is clearly to continue emphasizing those links where perceived as positive, and reap the benefits of high profile media coverage of such products such as vitamin A-enhanced rice and foods containing built-in vaccines. As mass-market genetically engineered commodity crops appear to be in for serious, long-term trouble, the industry appears to be looking toward a future of more specialized products.

Steps have already been taken in the direction of more specialized, value-added genetically modified (GM) crops. Early efforts included Monsanto's high-lauric acid canola, developed primarily for the cosmetics industry, and Zeneca's tomatoes with altered pectin to improve processing. These were both introduced in 1995 (Union of Concerned Scientists 2000). There have also been widespread reports of potatoes and other crops engineered to produce plastic-like polymers (see Gerngross 1999, *Nature* 17:541-3 for a critique). But probably the most active area of research today is on the genetic engineering of plants to produce specific proteins of interest to the pharmaceutical and chemical industries. This is a logical extension of the work pioneered by companies such as Genzyme in the US and PPL Therapeutics in Scotland, using livestock as "bioreactors" to produce chemicals of interest in their milk. But while animal-based production systems have proved expensive, and raise significant technical problems, not to mention growing animal welfare concerns and ethical debates over the cloning of animals, the use of plants as living "bioreactors" is being touted as a much more advantageous solution.

In recent years, many of the leading agbiotech and agrochemical companies—Monsanto, DuPont and Dow, among others— as well as a considerable number of smaller, more specialized companies, have begun developing plant-based systems for pharmaceutical and chemical production. A number of vaccine components

and monoclonal antibodies have been produced by tobacco, potato and corn plants on an experimental basis, and several of these products have begun clinical trials. One company, the Texas-based ProdiGene, has been collaborating with Stauffer Seeds to produce eleven different proteins in genetically engineered plants on a commercial scale. This represents a significant new development in plant biotechnology, and one that has heretofore escaped public scrutiny.

These new crops present many of the same potential environmental problems as other genetically engineered crop varieties, particularly if they are to be grown outdoors on a large scale. Most noteworthy are problems of cross-pollination, and unknown deleterious effects on insects, soil microbes and other native organisms. Further, we may soon see biologically active enzymes and pharmaceuticals, usually only found in nature in minute quantities—and usually biochemically sequestered in very specialized regions of living tissues and cells—secreted by plant tissues on a massive commercial scale. The consequences may be even more difficult to detect and measure than those associated with more familiar GM crop varieties, and could escalate to the point where those now-familiar problems would begin to pale by comparison.

This new technology also has potential public health consequences. As commercial grain distributors have proved unable to reliably sequester such a relatively well-characterized product as Aventis' Starlink corn, what steps could be reliably taken to prevent the accidental commingling of crops engineered for chemical production into the rest of the food supply? Proponents of this technology in the U.K. have already proposed ameliorating the high cost of purifying specific proteins from plants with income obtained by extracting food products such as oils, starches and flours (G. Giddings, et. al. 2000 / full ref. below).

The Rationale: Why use plants to manufacture proteins?

Proteins make up at least 50 percent of the dry weight of living cells, and are fundamental to all aspects of cellular structure and function, from providing structural integrity, to regulating biochemical reactions, including the processes underlying the expression of genes. As our understanding of protein function has increased, many industries have found commercial uses for proteins that are known to mediate specific cellular functions. Enzymes— proteins that catalyze chemical reactions—are used in a wide array of industrial processes, and numerous proteins that perform regulatory functions are now commonly used as pharmaceuticals.

Making proteins available for use outside living cells has often proved problematic, however. Manufacturers have continually sought the most efficient and reliable ways to extract these highly specialized products from their natural sources. Many of these substances only exist in specific living tissues, and those with the most specialized biological functions can only be found in minuscule quantities in living cells, often only under very exacting biochemical conditions. The extraction of

many known proteins, whether for commercial or research purposes, has proved a daunting task.

Molecular biology and genetic engineering have considerably expanded the range of available means to isolate usable quantities of specific proteins. First, the amino acid sequences of many useful proteins have been discovered, sometimes making it possible to synthesize the protein in the laboratory. Increased understanding of metabolic regulation has made it possible in some instances to induce higher rates of production of specific proteins in cultured cell lines, and methods of extraction and purification have also improved dramatically.

Initially, *E. coli* bacteria, with their very well-characterized processes of gene expression and regulation, were mobilized for this purpose. Human proteins such as growth hormone and insulin, and products such as recombinant bovine growth hormone (rBGH) are manufactured in this way. Companies such as Genzyme in Massachusetts and PPL Therapeutics in Edinburgh are splicing genes for proteins such as protease inhibitors into the embryonic cells of sheep and other livestock, and attempting to purify useful proteins from their milk when the animals reach maturity. Other companies are experimenting with genetically engineered chickens, hoping to extract pharmaceuticals from their egg whites (*Feedstuffs* 1999/*BusIndustry* database).

But animals present significant technical, economic and ethical problems. Product yields are often very low, production costs may be expensive, and there is a significant risk of contamination with pathogens such as viruses.

Recent advances in plant genetic engineering have raised the possibility of producing pharmaceuticals and other human and animal-derived proteins in plants. Researchers, working mostly in commercial laboratories, have engineered plants to produce vaccines, tissue-specific (monoclonal) antibodies, and a wide array of animal-derived enzymes, blood factors, neurologically active agents, and other useful proteins. One company, the Texas-based ProdiGene, is collaborating with Stauffer Seeds (a spin-off of Stauffer Chemical, and formerly a division of Novartis) to produce ten specific proteins in genetically engineered field corn, including vaccines, enzymes and a new protein-based sweetener. Extracting proteins from corn kernels may alleviate problems of product storage, shipment and purification that often arise in bacterial and animal models. Other companies are using tobacco and potato plants as containers for experimental "bioreactor" viruses, and one Virginia-based company (CropTech) actively advertises this technology as the saving grace for struggling tobacco farmers (Harr 1998/*Farm Progress*, at www.croptech.com/transgenic%20tobacco%20FP%2011%2098.htm).

It remains to be seen whether any compelling technological or clinical advantage will be obtained from these products. However, the biotechnology industry's public relations needs are readily apparent. In the November 2000 issue of *Nature Biotechnology*, Julian Ma of Guy's Hospital in London writes:

> "Indeed it is to be hoped that the eventual market release and safety evidence from GM plant vaccines might allay many of the safety concerns surrounding GM foods." (p. 1142)

The Problems: What is wrong with this picture?

Critics of genetic engineering have raised numerous concerns about the environmental consequences of large-scale production of GM crops. An increasing number of peer reviewed studies has validated concerns about cross-pollination of related crops and wild relatives, damaging effects on non-target populations of insects and arthropods, soil contamination via the secretion of transgene products from plant roots, and alterations in the populations and behavior of soil microbes, to name just a few of the impacts.

The Starlink corn scandal in the United States raises the further question of whether crops engineered to produce industrial chemicals and pharmaceuticals can be successfully isolated from the food supply. While Aventis quickly attempted to recall stocks of Starlink corn, it is clear that farmers and grain silos had already commingled this potentially allergenic corn with much larger quantities of approved varieties, as well as with non-GMO corn. There is still only circumstantial evidence that the particular variety of Bt toxin that is expressed in Starlink may be allergenic to humans, but in the case of plants that contain pharmaceuticals and other animal and viral proteins, the consequences may be much more severe.

The introduction into the food supply of byproducts from these new generation GM crops may indeed prove crucial to the commercial success of this technology, as the cost of purifying proteins from plant tissues is often quite prohibitive. Glynis Giddings and colleagues, from the Institute of Biological Sciences at the University of Wales, recently reviewed the purported benefits of GM plant-derived pharmaceuticals in the journal *Nature Biotechnology* (November 2000), and discussed ways of overcoming difficulties with extraction and purification:

> "An alternative approach is to cover the costs of purification with the income from the extraction of conventional products, such as meal, oil, or starch." (p. 1151)

Tony Laos, president of Stauffer Seeds, the company that has pioneered the commercialization of this technology, told a reporter that "The actual grain becomes a by-product in the protein production," thereby further suggesting that such products will find their way into the food supply. (Vacek, at www.sarahvacek.com/media/Amvalue-added.htm)

The problem of soil contamination has already been documented in the case of Bt toxin (Saxena, et al., 1999). In that case, biologically active quantities of the active bacterial toxin were found in soil samples for more than 9 months after the GM plant was harvested. In the next generation of GM plants, there are plans to take

commercial advantage of this phenomenon, a technique that has been termed rhizosecretion:

> "In this technology, transgenic tobacco plant roots submerged in hydroponic solution continuously secrete proteins at 3% total root secreted protein." (Hood and Jilka, 1999, at www.prodigene.com/publications/99-10-01_plant_based_2.html).

Rhizosecretion is being touted as an economical alternative to the chemical extraction of biologically active compounds (ihumans.com/news_comments_archive/plant_for_protein_prod.htm). If this indeed becomes a viable possibility in the field, how will secretion into agricultural soils be adequately controlled?

While many companies that are active in this field suggest that these specialized GM crops will be contained in greenhouses, or hand-harvested before pollination, it is clear that for many products, successful implementation of this technology will require very large-scale outdoor plots. For example, Carole Cramer of Virginia Polytechnic Institute, the founder of CropTech, told a reporter from *Farm Progress* that for some proteins, thousands or even hundreds of thousands of acres, planted at densities (in the case of transgenic tobacco) of 50,000 to 100,000 plants per acre, would be needed to supply the current market for these products. (Harr, 1998, op. cit.).

Concerns about the public health and environmental consequences of these crops are exacerbated by their wide range of very high-level biological activities. Products being actively researched for plant-based production include blood coagulants, proteases and protease inhibitors, growth promoters, neurologically active proteins, and enzymes that modify the structure and function of other biologically important compounds, as well as monoclonal antibodies and viral surface proteins potentially useful for vaccination. Large scale releases of antibodies and viral antigens may trigger unexpected allergic or autoimmune reactions in some people. Further, the purported benefits of plant-produced vaccines are cast in doubt by the well-documented phenomenon of oral tolerance: a concerted loss in vaccine efficacy that often follows the administration of antigens through a mucous membrane (Ma, op. cit., Mason and Arntzen/Croptech). Materials such as cholera toxin are often needed as cofactors (adjuvants) to increase the effectiveness of oral vaccines (ibid.). Contamination of pharmaceuticals with pesticide residues has also been identified as a significant problem for manufacturers (USU Biotechnology Center, 1999, at www.usu.edu/~biotech/extnews/extnew25.html).

The active collaboration between ProdiGene and Stauffer Seeds has already brought several products of this technology to market, and their products serve to highlight the potential hazards of plants engineered to produce commercial proteins. Stauffer is actively contracting with farmers to grow corn containing the genes for three or four enzymes, three vaccines, a protein-based sweetener, a proprietary "Therapeutic

Agent," and two other biologically active chemicals (www.staufferseeds.com/0404prod.htm). Three of their products, avidin, beta-glucuronidase and aprotinin (a protease inhibitor commonly used by surgeons), have been produced in sufficient quantities to be sold through a commercial chemical supplier, the St. Louis based Sigma Chemical Company (ProdiGene press release June 10, 1997, Olson 2000/Farm Industry News).

Avidin is a protein that occurs naturally in raw egg whites. While Sigma markets it for use in medical diagnostic kits, it is also used as an insect growth inhibitor and is being investigated as a next-generation biopesticide (USDA 2000, at www.ars.usda.gov/is/AR/archive/aug00/egg0800.htm). Avidin binds to biotin, an important B-vitamin, and prevents its absorption across the intestinal mucosa (Calzyme Laboratories catalog). It causes a type of vitamin B deficiency in some people who consume raw egg whites (Worthington Biochemicals catalog).

There are contradictory reports as to whether beta-glucuronidase was produced by Stauffer in 2000, but it appears to have been available from them for a number of years. This enzyme reverses a biochemical reaction that helps render irritant molecules soluble. This added solubility helps to facilitate the detoxification and elimination of compounds as diverse as hormones, antibiotics and opiates. In the presence of this enzyme, potential toxins are freed from the molecular complex that enables their proper excretion. One can only speculate on the consequences of elevated levels of such compounds being released into the open environment.

Stauffer's professed goal is to maximize production of these and other compounds via both foreign and domestic production of transgenic corn, allowing for three growing cycles per year. According to their web site, production is currently taking place in South America, the South Pacific, and the Caribbean, as well as within the continental U.S. (www.staufferseeds.com/0405econ.htm). As South America is a center of biodiversity for maize, Stauffer's (presumed) biosafety studies of the potential for disruption of indigenous wild relatives will be eagerly awaited.

Companies and Products—A Brief Summary

Below is a partial listing of key companies currently involved in this technology and some of their products:

ProdiGene/StaufferSeeds (www.prodigene.com, www.staufferseeds.com)

Avidin, Aprotinin, Beta-glucuronidase, Trypsin, "Enzyme No. 1" (identity is labeled 'confidential'), Laccase, TGEV (Transmissible Gastroenteritis Virus vaccine for swine), Hepatitis B Vaccine (human), LtB (human *E. coli* vaccine), "Therapeutic Product No. 1" (also labeled 'confidential'), Brazzein (a protein sweetener of West African origin). Preferred crop: field corn.

CropTech (www.croptech.com)

Human lysosomal proteins (glucocerebrosidase, iduronidase), human serum albumin, urokinase, sIGA/G (secretory monoclonal antibody hybrid), bacterial enterotoxins, hepatitis B virus surface antigen, Norwalk virus capsid protein, human insulin, glycoproteins. Preferred crop: tobacco. Several clinical trials are in progress. Solicits contracts with pharmaceutical companies for small-scale (non-field) production. Developing techniques to make plant-derived pharmaceuticals more compatible with human cells (www.biotech.vt.edu/outreach/biotech-times/5_98/pharming.html).

EPIcyte (San Diego, CA.)

Partnered with Dow Chemical to develop and produce monoclonal antibodies in plants. Five antibody products in development, using technology licensed from Scripps Research Institute. Working to develop plant-produced topical microbicides against HIV and herpes and a topical contraceptive. Goal is to produce 10,000 kg annually of plant-derived monoclonals (Potera 1999, at dev.asmusa.org/memonly/asmnews/apr99/topic2.html). In an independent effort, Dow is also working on a corn-derived "natural" plastic (Genetic Engineering News, Feb. 15, 2000).

Integrated Protein Technologies (Monsanto subsidiary, www.iptbio.com)

This subsidiary of Monsanto seeks to contract with various clients to produce commercial quantities of proteins in corn, tobacco and soybean plants. Promise capability of producing several metric tons of any appropriate protein within three years. Eight current projects focus on monoclonal antibody production, including a collaboration with Bristol-Myers Squibb; also industrial enzymes, pharmaceutical proteins and vaccines (USU Biotechnology Center, op. cit.) Uses purification technology from ProMetic BioSciences, via a collaborative agreement. Monsanto's DeKalb division is also working on corn that produces poultry interferon as a possible antiviral (www.staufferseeds.com/0704feed.htm), and the parent company has been involved in efforts to produce a polymer plastic called Biopol from wheat sugar (GEN 2/15/00). Monsanto's Agracetus division (Middleton, WI) is also involved in this technology.

Planet Biotechnology (Mountain View, CA.)

Seeking to commericalize technique developed at Guy's Hospital Dental School in London to use plant-produced secretory antibodies to prevent tooth decay. Antibodies against *Streptococcus mutans* are produced in tobacco and corn, and clinical trials (1998) suggest a potential for medium-term protection against dental caries. Developing antibody-based therapeutics for "infectious diseases and toxic conditions affecting oral, respiratory, gastrointestinal, genital and urinary mucosal surfaces and the skin." (Press release, 4/28/98) Therapies for intestinal pathogens including hepatitis virus, *Helicobacter pylori*, enterotoxigenic *E. coli*, and cholera.

Meristem Therapeutics (www.meristem-therapeutics.com)

An independent spin-off from the French seed giant Limagrain, with US

headquarters in San Francisco. Primarily engaged in contract production, with products including human hemoglobin, lactoferrin, laboratory techniques for controlling glycosylation. Tobacco is their primary vehicle.

Large Scale Biology Corp. (Rockville, MD., www.lsbc.com)

Enzymes, cytokines, human and veterinary prototype vaccines, produced in tobacco plants. Developing a patient-specific non-Hodgkins lymphoma vaccine. Collaboration with Dow in functional genomics; company VP for genomics is a former Monsanto plant molecular biologist.

Other players include **Protein Technologies, Inc.** (a division of DuPont), Cornell University's **Boyce Thompson Institute for Plant Research**, **SemBioSys** (Calgary, Canada), **Battelle Laboratories** (Columbus, Ohio and Richland, Washington), and **Applied Phytologics** (Sacramento, California).