Jan. 10, 2003

Food & Drug Administration

To Whom It May Concern:

Enclosed please find comments for Docket No. 02D-0324. Comments also e-mailed to FDA today.

Sincerely,

Bill Freese, Research Analyst
Friends of the Earth

02D-0324 C543

Comments on Draft Guidance for Industry

Drugs, Biologics and Medical Devices Derived from Bioengineered Plants for Use in Humans and Animals

Docket No. 02D-0324

by Friends of the Earth for Genetically Engineered Food Alert

Submitted to

Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20852

on January 10, 2003

Introduction

In July of 2002, Friends of the Earth (FoE) and Genetically Engineered Food Alert (GEFA) released the most comprehensive report to date on "biopharming" entitled ***Manufacturing Drugs and Chemicals in Crops: Biopharming Poses New Threats to Consumers, Farmers, Food Companies and the Environment***, a copy of which is appended as an integral part of this submission. Based on this report, we have concluded that the practice of engineering food crops to produce biopharmaceuticals and other compounds not meant for human food use poses too many risks – to human health, the environment and the economic interests of farmers and food companies – to be undertaken safely, whether outdoors or in "confined" systems. We believe that use of recombinant DNA technology for plant-based production of pharmaceuticals should only be pursued, if at all, in non-food crops or in plant cell cultures in strictly contained systems. Recombinant techniques, of course, have been widely used for 20 years to produce medically useful proteins in contained cell culture systems. These techniques can be used to produce most or all of the biopharmaceuticals envisioned for production in plants (though as detailed below, more careful testing is required even for these products). Alternative plant-production systems based on rhizosecretion (for example) can also be developed in strictly contained systems.

Adoption of our position by the FDA and USDA would make much of this proposed federal biopharm guidance document irrelevant. Thus, many of our comments seek to demonstrate the pressing need to prohibit biopharming as outlined above. Should the federal government continue to allow biopharmaceutical engineering to proceed, however ill-advised, the guidance document still has numerous recommendations that are inadequate or illogical in their construction. Our comments on these deficiencies should NOT be construed as an endorsement of biopharming contingent upon implementation of our recommendations.

Line number references (e.g. 479) refer to the guidance document. Relevant sections of FoE/GEFA's biopharm report, mentioned above, are cited as follows (e.g. FoE/GEFA 4.9.1).

Guidance not Adequate

A mere guidance document is not adequate to the task of regulating the practice of biopharming. As argued in a recent petition to the USDA, there is a pressing need for the USDA & FDA to implement state-of-the-art protective regulations and undertake a programmatic environmental impact statement with respect to genetically-engineered pharmaceutical-producing plant varieties[1]. If done properly, we believe such an exercise would lead the government to adopt our position as outlined above.

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[1] "Petition on Genetically Engineered Pharmaceutical-Producing Plant Varieties," submitted by Center for Food Safety on behalf of Genetically Engineered Food Alert, December 16, 2002.

The guidance document has many weaknesses. There are far too many vague recommendations couched in language such as "you should consider the use of" (e.g. 479, 533-34), "you may want to consider" (e.g. 489), "we recommend that you" (e.g. 602), or at best "[w]e strongly recommend" (e.g. 497). The frequent suggestions that industry consult with the FDA or USDA on a case-by-case basis are likewise not reassuring (e.g. 501-03, 560-61, 569-71, 634-36, 941, 955-56, 974). In both cases – weak recommendations and recourse to *ad hoc* consultation & rulemaking – the FDA and USDA reveal their failure to adequately assess and formulate regulations for the pertinent issue. This approach allows applicant companies far too much leeway to concoct novel and untested schemes that may fall completely outside of measures considered or recommended in the guidance. In fact, the FDA and USDA explicitly welcome such schemes at the outset of the guidance document (116-17):

> *"An alternative approach may be used if such approach satisfies the requirements of applicable statutes and regulations."*

For example, if a company were to develop some "alternative approach" to permit it to make "dual-use" of a drug-plant hybrid for both drug production and food/feed use (FoE/GEFA 4.3), the FDA/USDA would presumably give it an *ad hoc* assessment, without public or external scientific review. Because "alternative approaches" are most likely new to the regulatory agencies and require case-by-case consideration, they cannot be given the measured and careful assessment entailed by a formal rule-making process; they evade both external scientific and public review as well.

Because of the many unique risks posed by biopharming, it is simply unacceptable to address them on such a casual, case-by-case basis, at the level of individual permit conditions, or through loose "guidance" recommendations. It will perhaps be argued that the broad variety of plant-made pharmaceuticals (PMPs) and situations in which they are grown make it impossible to establish standards that are both strict and general. Yet strict, mandatory standards can be developed that apply to particular crops, particular classes of PMPs, particular growing situations, etc. Failure to do so reveals the unwillingness of the government to anticipate and squarely confront challenging, problematic issues and design adequate regulations to deal with them – particularly cross-contamination of food crops.

Excluded and Improperly Classified Compounds

The guidance apparently does not apply to transgenic plants engineered to produce research chemicals, industrial enzymes or other substances not intended for use as pharmaceuticals and/or not meant for human consumption (126-28). (If this interpretation is incorrect, the definition of "regulated product" should be modified to explicitly include such substances.) There are at least two obstacles here.

First, regulation of transgenic plants is improperly based on the "intended use" of the recombinant protein rather than its actual properties (FoE/GEFA 6.4.2). Thus, plants producing recombinant proteins intended for use as research chemicals can be grown outside of USDA's permit system and/or escape regulation by the FDA and/or EPA even if they possess pharmaceutical, insecticidal and/or known harmful properties (two examples are avidin and aprotinin, see FoE/GEFA, 4.5 and Appendices 2 & 3). "Regulated products" should be defined based on actual properties rather than intended uses.

The second obstacle is USDA's inconsistent, loosely applied classification system. Among the 300 some odd "phenotypes" used by USDA to classify recombinant, plant-produced proteins on its field trial website, one finds the catch-all category "novel protein," which provides no useful information about the nature of the protein[2]. In at least one case, the "novel protein" category is being applied to a substance (laccase, e.g. Permit 02-113-09n) that is explicitly intended for use as an "industrial enzyme" (see FoE/GEFA 4.10.2), another phenotype employed by USDA. In another case, it appears that a PMP now properly classified as "pharmaceutical" (aprotinin) was formerly listed as a novel protein (FoE/GEFA Appendix 3). A third example is the confusing use of the "antibody" phenotype, which USDA has made a category separate from, rather than a subset of, "pharmaceutical protein."

APHIS should precisely define, reformulate as necessary, and consistently apply the phenotypes such that they fully reflect the actual properties of the protein rather than merely its intended use. (This may mean applying several categories to a single recombinant compound.) The various phenotypes should be definitely assigned to either the notification or permit system. In addition, APHIS, the FDA and EPA should formulate clear and detailed procedures for the review appropriate to each phenotype, including which agencies take part in the review and the elements of that review. The review should take place before any field trial permit/notification is issued by APHIS. The information on phenotypes outlined above (assignment to notification or permit, elements of review required for each) should be made available on APHIS's website.

The novel protein category should be abolished because it provides no useful information and can be used to avoid specifying even the general nature of the protein (and APHIS/BRS provides the public with little enough information as it is). Antibodies should be made a subset of pharmaceutical proteins.

Recombinant plants that produce industrial enzymes and other substances not meant for human consumption that are nevertheless not covered under the current definition of "regulated product" should be: 1) Included in the scope of this guidance or subsequent regulations that supplant it; and 2) Subjected to USDA's permitting process rather than the weak notification system.

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[2] Strictly speaking, ALL plant-produced recombinant proteins are novel, or should be considered so until full sequencing demonstrates otherwise.

The Illusion of Zero Tolerance

Host plants (232-253)

If biopharming is to be permitted at all, APHIS/FDA should at the very least ban the use of food crops, which pose risks of contaminating the food supply through cross-pollination, volunteer growth the following season and other modes of seed dispersal. Corn and canola, in particular, should be banned, due to their high propensity for cross-pollination (FoE/GEFA 5.4).

Isolation of biopharm crops (489-90)

> *"For such plants that outcross, you may want to consider growing them in regions of the country where little or none of its food/feed counterparts are grown."*

While this measure would help reduce the risk of food/feed contamination via cross-pollination, it would do nothing to guard against the sort of volunteer contamination responsible for the ProdiGene episode in Nebraska, where biopharm corn volunteers contaminated soybeans grown on the same plot the following season. Other ways such contamination could occur are spillage of biopharm seed, biopharm seed carried to conventional fields in farm equipment, movement of biopharm seed by animals, and extreme weather events. The Royal Society of Canada notes that these many modes of seed dispersal may well pose a greater risk of contamination than cross-pollination (FoE/GEFA 7.3.2)[3]. In addition, because the most popular biopharm plant, corn, is grown on 70-80 million acres across the country, it may be difficult to find sites which provide both adequate isolation from conventional corn and adequate growing conditions (soil quality, weather, adequate water, etc.). This problem will become more acute for biopharm/industrial crops growing high-demand compounds that necessitate substantial acreage in the thousands to hundreds of thousands of acres (FoE/GEFA 4.8, 4.10.2, 7.2.1)

Responsibility for confinement measures (456-63)

> "Regardless of whether the bioengineered pharmaceutical plants are grown and/or processed by you or on a contractual basis by other persons, manufacturing controls are your responsibility and should be documented clearly

[3] "Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada," An Expert Panel Report on the Future of Food Biotechnology, The Royal Society of Canada, p. 123. See http://www.rsc.ca/foodbiotechnology/indexEN.html.

in standard operating procedures (SOPs), Outlines of Production, or other records, as appropriate..."

The food and grain supply have been contaminated several times now with recombinant proteins unapproved for human consumption. In at least two of those cases, breaks in the "chain of responsibility" for "manufacturing controls" was at fault. In the case of StarLink, the EPA delegated responsibility to Aventis, which in turn apparently relied on seed companies (chiefly Garst) to inform farmers of planting restrictions and the prohibition against food use. Many farmers never heard of these restrictions, or were given false information by Aventis and/or Garst (apparently to increase StarLink seed sales).[4] In the recent case of biopharm corn contamination of soybeans in Nebraska, on-the-ground responsibility for monitoring and preventing contamination was apparently divided amongst at least four players: USDA inspectors, ProdiGene, an agricultural consultant hired by ProdiGene to supervise the trial, and the farmer contracted by ProdiGene to actually grow the biopharm corn. A USDA inspector discovered volunteer growth, communicated this to ProdiGene, which in turn contacted the ag consultant, who didn't get the job done. A fifth player in this case was Nature. An unexpected hail storm reportedly opened up the soybean canopy, allowing light to reach the soil and trigger the sprouting of biopharm corn seeds left over from the previous year's harvest.[5]

Whoever is legally responsible, it is clear that in practical terms, responsibility for confinement measures is scattered among many players. This is a sure recipe for continued contamination episodes. It is difficult to envision a system, however, in which this chain of responsibility is tightened and shortened enough to do more than reduce somewhat the risk of contamination, especially with continued reliance on contract farmers.

Phenotypic markers (481-82)

The guidance recommends that companies consider use of phenotypic markers (e.g. novel color or leaf pattern) when using food/feed crops to generate biopharmaceuticals in order to distinguish them from their conventional counterparts. It is true that an altered phenotype might help prevent those in the know from inadvertently mixing the biopharm crop in the food supply. If this phenotypic trait were reliably transferred and expressed in progeny in cases of cross-pollination, it might also help those in the know to single out biopharm volunteers resulting from inadvertent biopharm-conventional crop crosses. However, the utility of phenotypic markers would be limited to those aware of the connection between pharmaceutical and phenotype. Farmers, grain buyers, elevator operators, food processing workers, and others in the grain/food supply

[4] Ryberg, W. "Growers of biotech corn say they weren't warned: StarLink tags appear to indicate it's suitable for human food products," Des Moines Register, Oct. 25, 2000. See also: Freese, B. "The StarLink Affair," a report submitted to the EPA's Scientific Advisory Panel on behalf of Friends of the Earth, 2001, sections 10 & 11, Appendix VII. See www.foe.org/safefood/starlink.pdf

[5] Gillis, J. "Farmers Grow a Field of Dilemma: Drug-Making Crops' Potential Hindered by Fear of Tainted Food," The Washington Post, Dec. 23, 2002.

chain who are unaware of the connection would likely not be deterred from accidental misuse.

On the other hand, phenotypic markers greatly increase the risk of ***intentional*** misuse, because a malicious person who learns that a crop expressing a particular pharmaceutical substance has a particular phenotype could use this information to illegally harvest and then disseminate, cultivate or otherwise make illicit use of the crop. Despite the great emphasis on secrecy by government and industry with respect to all aspects of biopharming, it would be difficult to keep such knowledge of phenotypic markers secret (FoE/GEFA 7.4.3).

In short, phenotypic markers would *marginally* reduce the risk of inadvertent contamination, but greatly increase the chances of intentional misuse (especially given the industry's preferred practice of anonymously planting biopharm plots with no fences or other security measures).

Identification and security of biopharm plantings

(532-34)

Identification of biopharm plots poses the same intractable dilemma as phenotypic markers. Identifying plots may reduce the risks of inadvertent misuse/contamination, and is of course also needed to alert neighboring farmers and the public to the risks of contamination, but it would increase the chances of intentional misuse. Enclosure of biopharm plots with high-security fences and the provision of alarms, floodlights, guards, etc. to prevent theft/illicit misuse would be extremely expensive, thus eroding the cost advantage that is the primary driving force behind biopharming. Such security measures would also be impractical for all but the tiniest plots. Compounds that only require small plantings to meet anticipated demand are likely to be biopharmaceuticals that are active at very low doses – that is, potent biopharmaceuticals (e.g. growth factors – see FoE/GEFA 4.9) – that definitely should not be grown out-of-doors at all due to potential health impacts on farmers from exposure during harvesting/processing and consumers from accidental contamination of food products. This is yet another reason that biopharmaceuticals should only be produced in truly contained facilities, such as pharmaceutical plants.

Tests for biopharmaceutical gene and protein

(272-74; 497-501)

> *"We strongly recommend that you have tests available that can detect the presence of the target gene and the protein product in the raw agricultural commodity."*

A mere recommendation, however strong, is totally unacceptable here. In fact, in the interests of agency independence from the regulated company, the USDA or FDA should

develop its own DNA primer sets and protein detection tests for each and every PMP before planting[6]. Failing this, the agencies should ***require*** that the company not only develop such tests, but turn them over to agency officials, who would then verify their accuracy and sensitivity and employ them for regular inspection rounds to test for possible contamination of any cross-compatible neighboring plants or weeds.

However, one must be realistic about the capability of protein tests. Expert advisors to the EPA who examined the StarLink issue in great depth decided that the ELISA assays used to measure Cry9C in processed foods were unreliable because food processing could denature or degrade it into a form not detectable by the assay.[7]. Likewise, it will probably be difficult or impossible to accurately measure biopharm proteins that slip into processed foods through contamination episodes. This would seem to make it impossible to set enforceable tolerances (FoE/GEFA 7.4.1).

Biopharm products containing viable seeds (649-50)

Due to high risks of uncontrolled propagation, the FDA should prohibit the commercialization and distribution of biopharm products containing viable seeds.

Dedicated versus dual-use equipment (732-34; 746-47)

The guidance merely recommends the use of dedicated equipment. Alternately, the applicant is encouraged to develop "equipment-cleaning procedures" and to document other uses of the equipment. Yet no equipment-cleaning procedure will be adequate to prevent carryover of biopharm seeds (e.g. corn) or completely eliminate engineered viral vectors (e.g. TMV). The editors of Nature Biotechnology ask the rhetorical question:

> *"Can we reasonably expect farmers to [clean] their agricultural equipment meticulously enough to remove all GM seed?"*[8]

The answer, of course, is no – especially when plantings increase from a few acres to many thousands (FoE/GEFA 7.3.2). It should also be noted that the need for dedicated equipment, as well as other gene containment measures and expensive precision agriculture techniques, will price biopharming beyond the means of less wealthy, small and medium-sized farmers (FoE/GEFA 7.3.4).

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[6] A similar recommendation was made in the aftermath of the StarLink debacle. See "Assessment of Additional Scientific Information Concerning StarLink Corn," FIFRA Scientific Advisory Panel, SAP Report No. 2002-09, p. 39. The FDA had ***not*** developed such tests for StarLink, was caught flat-footed by the StarLink contamination of the food supply, and had to call in Aventis CropScience to help it develop such tests.

[7] Ibid, pp. 12-14.

[8] "Going with the flow," editorial, Nature Biotechnology, Vol. 20, No. 6, June 2002.

Transfer and storage conditions (749 – 772)

The guidance requests only "confinement" of the harvested plant material during harvest, an implicit admission that complete "containment" is impossible.[9] The guidance also merely recommends that the biopharm plant material container be labeled. This should obviously be a requirement. There is no provision for dedicated silos or other storage containers, despite the obvious risk of food crop contamination with dual-use storage facilities. Dedicated storage facilities should be made a requirement.

Greenhouse growth (428-433)

The guidance exempts biopharm plants grown in "an enclosed building (e.g. greenhouse)" from an APHIS permit because they are generally considered to be "confined," yet at the same time admits that "control measures" must be in place "to eliminate the spread of pollen or seeds outside of the facility." It is entirely unclear how APHIS could ensure use of adequate "control measures" if such indoor trials do not require APHIS permits. Such indoor trials – especially if they make use of food crops – should require USDA permits and inspection visits.

Potential Health Impacts of Contamination Ignored

Regulatory Responsibility (177-213)

While contamination of food-grade crops with biopharmaceuticals is widely regarded as inevitable by leading experts (FoE/GEFA 4.7, 5.4, 6.3.3, 6.4.5), no one is taking responsibility for assessment of the potential human health impacts arising from such episodes. And it should not be presumed that any contamination that occurs would be intermittent or at low levels, the faulty assumption upon which the recent Office of Science and Technology Policy (OSTP) directive[10] is based. Both the risk of contamination and the extent of exposure increase greatly as plantings grow from field

[9] "Confinement" has come to replace the formerly-used "containment" in all aspects of GE crop regulation in order to reflect the fact that complete containment of gene flow is impossible.

[10] "Proposed Federal Actions to Update Field Test Requirements for Biotechnology Derived Plants and to Establish Early Food Safety Assessments for New Proteins Produced by Such Plants," Federal Register, Aug. 2, 2002. As presently written, the OSTP directive does not apply to biopharm plants, but we can expect some such scheme to be presented for PMPs, given the inevitability of contamination and the desire of the biotech & food industries to avoid the associated liability.

trials of a few acres to commercial plantings of many thousands. Clearly, the USDA has no competence or regulatory authority to undertake health assessments, despite the fact that it tried to do so once, tucked away in an environmental assessment of trichosanthin-producing tobacco (FoE/GEFA Appendix 4).

If open-air biopharming is not stopped (the best solution), the Food and Drug Administration must step up to the plate and conduct a thorough review of potential human health impacts before any more such plants are allowed to be planted. The review should cover at least two distinct areas: 1) Oral exposure (inadvertent ingestion by consumers); and 2) Inhalant/dermal exposure during growth, harvesting and processing (farmers, farm-workers, processing workers). A review of the available literature on the native form of the substance should be supplemented by thorough, independent studies on the potential health impacts of the plant-grown version (not a bacterial surrogate, which can be different, especially in terms of immunologic and allergenic properties). This review should be conducted before any planting is allowed.

Failing a complete ban on open-air biopharming, the USDA/FDA should prohibit cultivation of transgenic plants producing certain (classes of) substances for which appropriate data are not available or which are found to pose risks based on a thorough review. And it should be emphasized that the "early food safety assessment" procedures outlined in the OSTP directive (see footnote 10) must NOT be extended to field trials of biopharm plants[11], because they are not nearly comprehensive enough to detect potential allergenic or toxic effects of PMPs or food-grade crops contaminated by them. These superficial assessments are being promoted mainly as a means to absolve the biotech and food industries of liability for contamination episodes.

The government's performance thus far bodes ill for the future of biopharm regulation. Consider the following facts:

1) **Known health effects of current PMPs ignored:** At least three biopharm/industrial compounds grown for many years in biopharm corn are known to have deleterious effects on human health. Avidin is a corn-grown insecticidal protein that is known to cause Vitamin B deficiency upon ingestion (FoE/GEFA 4.5.1, Appendix 2). Aprotinin is a blood-clotting protein from a group of substances known to cause pancreatic disease in animals (and probably humans) upon ingestion (FoE/GEFA 4.5.2, Appendix 3). Trypsin is an inhalant allergen known to cause occupational asthma in workers exposed to it, and thus could pose a similar risk to farmers and farm-workers who harvest it (corn dust & pollen) (FoE/GEFA 4.10.1). As far as we know, the FDA has failed to assess these or any other biopharm proteins for potential health impacts.

[11] The OSTP policy directs the USDA, FDA and EPA to establish voluntary procedures by which companies developing biotech plants can obtain a rubber-stamp approval (a.k.a. "early food safety assessment") alleging that novel biotech proteins in GE plants grown in field trials are safe. If implemented, this would permit contamination of food-grade crops with still largely untested GE field trial traits. While the OSTP policy does not apply to biopharm plants/PMPs, one can expect some such policy to be announced soon, given the impossibility of zero tolerance.

2) **No restrictions on host plants:** There have been no regulations to restrict the choice of host plants for biopharm production to non-food crops. In fact, USDA has permitted one of the worst crops in terms of contamination (promiscuous corn) to become by far the favorite crop choice for biopharmers (70% of biopharm field trials have made use of corn) (FoE/GEFA 5.4.1, 6.2);

3) **Deficient oversight:** The USDA does not conduct ANY inspection of 90% of field trial sites involving plants grown under its "notification" system, which includes plants engineered with industrial chemicals (personal communication, James White, USDA). The USDA allowed 500 bushels of biopharm corn-contaminated soybeans to get mixed with 1,000 times that amount of clean soya in an Aurora, Nebraska grain elevator, one step away from the food/feed chain, then had the brazenness to declare this a regulatory success.

Exposure to biopharm proteins through dual use

(563-579)

While the guidance recommends "disposal [of biopharm plant material] in a manner to ensure that the material will not enter the human or animal food chain," – it then immediately creates a loophole – "unless you have specifically consulted with FDA for the use of this material in food or feed products." This loophole is the only reference in the entire guidance to an extremely troubling aspect of biopharming – dual use of biopharm crops for both drug and food/feed purposes (see also FoE/GEFA 4.3).

First of all, we should recognize that what is presented here as an exception to the general rule of disposal would likely become the norm for most biopharm and industrial crops, for several reasons:

1) **Dual-use – an offer too good to refuse:** Because dual-use would offer companies substantial benefits – both avoidance of disposal expenses and profit from sale of biopharm plant byproducts into the food/feed chain – in many cases they will aggressively lobby the FDA to approve dual-use under this loophole;

2) **Mountains of waste:** This is especially true for high-volume compounds requiring, say, thousands of acres to meet demand. For example, contraceptive corn would require tens of thousands of acres (FoE/GEFA 4.8), while laccase corn (according to ProdiGene's projections) could be planted on 200,000 to 2 million acres (4.10.2). To give an idea of the magnitude of the problem – and the cost savings/profit from dual-use versus disposal – consider that just 1,000 acres of corn yield over 8 million pounds of corn kernels alone, not counting other parts of the plant. And since the extracted biopharm protein will represent an insignificant proportion of the corn kernel, nearly 8 million pounds of kernel byproduct, some of it suspended in column wash solutions and such, would have to be "treated to inactivate the regulated product," and then disposed of. How much will this cost? On the other hand, how much profit could be made by diverting millions of pounds of byproducts into the food and feed chain?

3) **Approval of dual-use:** We will presumably be told that dual-use will never be permitted without studies to demonstrate complete extraction of the biopharmaceutical and/or no adverse effects on animal/human health from any biopharmaceutical residues that remain in byproducts. Yet this is mere speculation. The FDA says absolutely nothing about criteria to be met for dual use in the guidance, preferring to deal with this huge issue on its own undisclosed terms on an *ad hoc* basis. Even if a laboratory or pilot-scale processing study should demonstrate reasonably complete extraction, should consumers and farmers depend on biopharm processors to consistently remove such potentially dangerous residues from materials entering the food and/or feed supply? To take one scenario. Use of biopharm corn byproducts for ethanol production would also generate corn gluten, consisting mainly of corn proteins, which might then be sold into the feed and food chains. Any unextracted biopharm protein residue would be concentrated in gluten.

4) **Other biopharm plant material:** It is unclear whether or not the guidance even addresses the disposition of those parts of the biopharm plant that do not enter into the purification process.

> *"In-process wastes (e.g. column wash solutions, diafiltration solutions, etc.), rejected in-process material, and* ***residual source plant material from the purification process*** *should be treated to inactivate the regulated product prior to disposal, as appropriate." (my emphasis)*

In this sentence, the phrase in boldface could be interpreted to apply only to those parts of the plant that are processed for extraction of the biopharmaceutical. In the case of corn, companies will normally process only kernels, not stalks, leaves, roots, etc. (If this interpretation is incorrect, the guidance should be amended to ***explicitly*** require harvest of, and inactivation of the regulated product in, ALL biopharm plant tissues.) If this interpretation is correct, the USDA/FDA need to address the troublesome issue of the biopharm protein present in millions of pounds of non-processed crop residues (see "'Tissue-specific' promoters" below, as well as FoE/GEFA 5.6.3). Will they be incinerated, composted? Will there by any provision for inactivation of the regulated product in such residues?

"Tissue-specific" promoters (485-87)

Use of so-called "tissue-specific" promoters is recommended to "reduce the likelihood of unintended exposure." More careful scientists employ the term "tissue-preferred" promoter in recognition of the fact that expression of the target protein is seldom or never limited to the target tissue; as even ProdiGene admits: "some expression may occur in other parts of the plant." There is also evidence that varying cellular and environmental conditions can reduce the tissue specificity of a tissue-preferred promoter (FoE/GEFA 5.6.3). USDA/FDA are encouraged to adopt the more accurate

term "tissue-preferred promoter" in place of the misleading "tissue-specific promoter," and not to rely on this mechanism as a means of preventing unintended exposure.

Viral-vectored transfection systems (341-369)

Viral-vectored transfection systems should not be permitted at all for biopharmaceutical production in plants due to our vast ignorance of viruses in general, their easy mutability, and the potential for infecting a related food crop with the biopharm gene. This latter consideration applies particularly to the tobacco-tobacco mosaic virus (TMV) system most commonly used in biopharm experimentation, since TMV is known to infect solanaceous family relatives of tobacco such as tomatoes, peppers, eggplant and potatoes, as well as numerous weeds. (For a detailed assessment of a biopharm field trial involving TMV-vectored infection of tobacco with the toxic protein trichosanthin, see FoE/GEFA Appendix 4.)

At the very least: 1) No viral vectors for which the "gene(s) involved in vector transmission" (356) is/are unknown should be permitted; and 2) No viral vector which has not been tested thoroughly and found negative for potential "synergistic or transcapsidation interactions with other viruses" in laboratory situations should be permitted.

Potential Health Impacts on Farm & Processing Workers Ignored

The guidance has nothing to say about measures to protect farmers and farm-workers who will be exposed to biopharmaceutical proteins through inhalation of crop dust & pollen, skin contact and ingestion (FoE/GEFA 7.7). This is not surprising, since the USDA and FDA have apparently given no thought at all to farm & processing workers. Amazingly, the entire 240-page transcript of the two-day "Plant-Derived Biologics Meeting" in Ames, Iowa – the major meeting held in April of 2000 to gather information to help formulate this guidance document – contains only a single brief discussion of possible health risks to farmers. An FDA official asks an industry representative whether his company would inform a contract farmer of a [potentially dangerous] PMP he is growing, "or would that be a problem?" (see FoE/GEFA 7.7 for more on this). Here's the situation: Government regulator, charged with protecting public health, timidly asks the regulated company whether it will inform a farmer of a potentially dangerous product the company has put into his/her hands. Unfortunately, the timid attitude adopted by this FDA official (who is to be commended for at least raising the issue!) is surpassed by the guidance document, whose silence on farm and

processing worker health speaks more loudly than any words about how little farmers' health means to government regulators, including those at the USDA whose job is supposedly to promote and protect their interests.

There is also no evidence to suggest that USDA or FDA has bothered to consult with farmers or representatives of genuine farmers' groups (as opposed to agribusiness lobbyists) about biopharming. There was not a single farmer's voice at the Ames meeting mentioned above.

In the interests of farm & processing worker health, companies should be required to:

1) Disclose the identity and any known harmful properties of the biopharmaceutical or industrial compound to farmers, farm-workers, processors, and others who will come into contact with it before any contracts are signed or exposure has occurred;

2) Provide all farm and processing workers with any necessary protective equipment, in line with government-approved standards, adequate to protect them from any adverse health impacts associated with the given compounds;

3) Contract independent and qualified health professionals to test all farm and processing workers who come into contact with the PMP for any adverse health impacts, including immunogenic or allergic reactions and toxic effects.

Such testing is necessary to protect front-line agricultural and processing workers, and it could have further benefits. Health impacts in these high-risk, high-exposure groups can signal potential risks to consumers. Such information could have proven valuable in the context of the StarLink corn debacle. Expert advisors to the EPA requested several times that seed company workers who grew and sold StarLink corn (from Garst Seed Company) be tested for allergies to Cry9C. Besides its obvious benefit to the workers, such testing would have helped the advisors determine whether Cry9C posed a threat to consumers. Neither Aventis nor the government saw fit to do this testing[12], and it is still uncertain whether Cry9C is a food allergen.

To take one contemporary example (FoE/GEFA 4.10.1). Trypsin (an industrial enzyme) was reportedly grown in hundreds of acres of corn in 2002, despite the fact that the conventional version is known to cause occupational asthma. The USDA and FDA, however, have refused to recognize the potential risks of trypsin exposure, refused to make an assessment of potential farm-worker health impacts. Apparently, the agencies prefer to let companies conduct uncontrolled experiments on their workers and contract farmers. Trypsin is just one of many industrial enzymes that may pose similar threats and which are anticipated to be grown on thousands to millions of acres (FoE/GEFA 4.8, 4.10.2, 7.2.1).

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[12] Freese, B. "The StarLink Affair," op. cit., section 7.2 (see footnote 4)

Economic Impacts of Biopharming on Farmers & Food Industry Ignored

No official venues for consideration of economic impacts

Unfortunately, there does not appear to be any official government venue for consideration of the economic impacts of biopharming – in particular, the economic consequences of inevitable contamination episodes – on American farmers. This is totally unacceptable. Instead, this vitally important issue is left to be thrashed out in the political arena, with financially-interested industry trade groups and woefully ill-informed politicians making decisions that could – and almost certainly will – have billion-dollar liability and export implications for American farmers and food companies. Likewise, there has been no official forum in which farmers – as opposed to agribusiness lobby groups – can bring their unique, on-the-ground perspectives to bear on the real risks of biopharm crops contaminating the food supply.

The USDA is urged to hold a high-level public forum on biopharming that addresses not only containment, consumer health & environmental issues, but also the question of the economic impacts of this enterprise on American agriculture, the food industry, and small/medium-size farmers. Such a forum should include not only scientists, health specialists, economists and agronomists, but also environmental/food safety advocates and, most importantly, farmers and representatives of agricultural organizations (such as American Corn Growers Association, National Farmers Union and National Family Farm Coalition) that actually represent small and medium-size farmers.

Survey attitudes of affected groups and conduct economic analysis of impacts of biopharming

> "...measures should be in place to ensure that there is no inadvertent mixing of the bioengineered plant material with plant material intended for food or feed use." (268-270)

This is one of many statements in the guidance upholding the illusion of zero tolerance. In fact, the only way to ensure the zero tolerance implied in this statement is to ban open-air biopharming, particularly in food crops. If USDA/FDA choose not to do this, the agencies should at the very least initiate an honest, wide-ranging public dialogue to ascertain the attitudes of the public, the farming community, the food industry, public

interest groups, etc. towards the inevitable episodes of biopharmaceutical contamination of the food supply that will occur with continuation of current policies.

The agencies should also conduct extensive consultations with grain handlers and traders in relevant crops – both American and foreign (particularly those in important export markets) – to the same end. This information should be used to develop detailed estimates of food company liability, crop export losses, economic impacts on the farming community, government expenditures (= taxpayer subsidies) for needed interventions (e.g. the USDA's purchase of StarLink-contaminated seed stock cost taxpayers tens of millions of dollars), and other adverse effects of such contamination episodes, which estimates should be made public. Such analyses could employ data generated on the continuing StarLink contamination episodes as a reference. (FoE/GEFA 7.4 & 7.5).

Biopharming will inevitably harm American agriculture and public trust in the food supply, but without honest dialogue and modeling of this sort, the magnitude of such harm will undoubtedly be greater than it would otherwise be.

Control and Liability Issues (518-537)

(See also "Responsibility for Confinement Measures" above (456-63))

> "You must ... have control over the growing process from planting through harvesting and over the disposition of remaining crops and/or crop residue and, if required, over the subsequent use of the field if for growth of food or feed or as a pasture during subsequent seasons."

The diffusion of on-the-ground responsibility for the growing process among many players (discussed under "Responsibility for Confinement Measures" above) creates numerous opportunities for communication breakdown, misunderstandings, and intentional deception, any of which can easily result in contamination episodes. At present, most biopharm field trials are conducted by contract farmers with only occasional visits by company officials, and few if any inspection visits by government inspectors. This situation cannot by any stretch of the imagination be described as "control over the growing process" by the company. Once again, the USDA/FDA show no understanding of the real world of farming, human fallibility, conflicts of interest, weather, etc., preferring the legal fantasy of "control" to the facts on the ground, which completely belie the notion that biopharming is controllable.

Therefore, the phrase "have control over" should be replaced by "bear liability for," so that at the very least contract farmers will not be held liable for the inevitable contamination mishaps that will occur, no matter how careful the farmer's stewardship.

Biopharming Poses Novel Threats to Drug Safety

Use of pesticides, herbicides, fungicides & other agricultural chemicals (699-717; 943-956)

Biopharming introduces a truly novel threat to the world of pharmaceutical production: the potential for residues of toxic agricultural chemicals in the ***drug*** supply. Never before have pharmaceutical users been confronted with the prospect of eating, applying or injecting a drug laced with pesticides, herbicides or fungicides. What does the guidance have to say about this important subject?

Information requested by FDA:
1) What pesticides, etc. the company plans to use and any limits on such use
2) What pests are expected

Recommendations made by FDA:
3) Develop standard operating procedures for recording pesticide applications
4) Pest-control measures should be in accordance with good agricultural practices
5) Only EPA-approved pesticides, etc. should be used
6) Companies should establish tolerances (i.e. maximum allowable levels) for "any pesticide, herbicide, and/or fungicide residues anticipated to be present, justify the safety of those amounts ***under conditions of anticipated use of the pharmaceutical***, and demonstrate that the final product does not exceed those limits" (my emphasis)
7) Applicants should check with EPA "if you have questions regarding the use or safety of pesticides..." (FDA helpfully cites the EPA's Pesticide Product Information Service webpage)

This paltry guidance does almost nothing to proactively protect pharmaceutical users from toxic residues in drugs. To understand this, consider the following:

Greater incentives for toxic chemical use on biopharm crops

First of all, biopharm crops will be extremely valuable, in some cases worth several million dollars per acre to the biopharm company.[13] This value creates strong incentives for protecting the crop in the field and in storage from insect pests, mold infestation, disease and competing weeds by whatever means possible. A contract farmer or biopharm company that relies on biopharming for a substantial part of his/her/its income will be especially anxious to ensure that the crop is not rendered unacceptable

[13] It should be remembered that contract farmers will likely receive very little of this value. For instance, ProdiGene has offered contract farmers at most 40% above commodity prices (about $1/bushel) for biopharm corn, and will not even guarantee this tiny premium.

due to insect damage, contaminating mold or mold toxin, etc. Thus, all other things being equal, pesticides, herbicides and fungicides are more likely to be applied to biopharm crops than to lower-value food & feed crops.

Diffusion of on-the-ground responsibility opens door to misuse
The diffusion of responsibility for biopharm crop production among company officials, contract farmers and agricultural consultants – and the dearth of government oversight – open up numerous opportunities for unprescribed use of these chemicals. If an unprescribed pesticide is applied, it may not be eliminated during extraction and purification.

Many ag chemicals implicated in cancer and/or hormonal disruption
Abundant research has shown that many registered pesticides are proven or suspected carcinogens; more recent studies have identified at least 56 pesticides as endocrine disrupters[14], which can have potent effects on brain & sexual development, the immune system, thyroid function, etc. at extremely low levels.

Injected drugs a particular concern
Pesticide residues are of particular concern for PMPs intended for injection. For one, pesticide residues injected into muscle tissue or infused directly into the blood stream are more likely to be active at far lower doses than if consumed or applied dermally, because they bypass the partial protection afforded by gastrointestinal tract or skin. In addition, there has likely been little research done on the effects of most pesticides upon injection or infusion, since exposure to agricultural chemicals in foods/crops is limited mainly to the oral (consumer) and dermal/inhalant (agricultural worker) routes.

Synergistic effects unstudied, ignored
Finally, pesticides residues might have synergistic effects with each other, or with the PMP.

Given these serious and in many cases ***novel*** risks, the guidance is ridiculously weak. Some questions that need to be answered:

1) Why does our Food and Drug Administration defer to the Environmental Protection Agency to provide applicants with information about the potential risks of toxic chemical residues contaminating ***drugs***, especially drugs meant for injection? Is the EPA qualified to give guidance on pesticide-laced biopharmaceuticals? (point 7 above)

2) Doesn't the FDA find ***any*** EPA-approved pesticides too risky for biopharmaceutical crop use, especially those with hormonal effects? (points 1 & 5) If so, why aren't they prohibited?

[14] See www.ourstolenfuture.org/basics/chemlist.htm

3) Of what relevance are "pest-control measures ... in accordance with good agricultural practices for the growth of ***food*** crops..." to the issue of pesticides residues in ***drugs***, especially injected drugs? (point 4)

4) Since when has private industry been put in charge of establishing tolerances for pesticide residues? (Obviously, EPA-prescribed tolerances for pesticide residues on ***foods*** cannot be automatically adopted for residues in ***biologics***.) (point 6)

5) What studies will the FDA require from companies to "justify the safety of those amounts [tolerances] ***under conditions of anticipated use of the pharmaceutical***," especially when the intended route of administration is parenteral? Will human experiments be conducted to determine the "safe" levels of injected pesticides?

6) What sort of inspection/testing regime will FDA establish for pesticide residues in biologics? (points 3 & 6)

Once again, the FDA's guidance raises more questions than it answers. Instead of a careful and thorough assessment of the novel issue of pesticide residues in biologics, the agency apparently prefers to give industry free reign to do what it wants, delegate the most difficult questions to a sister agency (EPA) with no experience in the field of drugs, and to bungle along with it usual *ad hoc*, case-by-case assessments of industry-formulated schemes for both biopharm crop pesticide use and removal of pesticides from PMPs.

Characterization (785-808)

The applicant should be required to fully characterize the nucleotide and amino acid sequences of the biopharmaceutical gene and its protein product ***as introduced into the plant genome and expressed by the plant.*** These sequences should be compared to the native versions of the gene/protein (for plant-made "animal" or "human" biologics). Identification of the insertion site in the plant genome, and characterization of plant DNA near the insertion site, should also be required. Non-targeted profiling techniques to detect the levels of a wide range of plant constituents (preferably, all) should be undertaken as well, especially for edible biologics. This information could prove to be vital for understanding any adverse, unintended effects of the PMP (FoE/GEFA Appendix 1).

Considerations for testing (896-990)

The FDA should under no circumstances allow the use of surrogate versions of the bioengineered PMP generated in organisms such as bacteria for the purposes of animal testing or human (pre-)clinical trials, due to the potential for important differences (e.g. immunologic, allergenic) between surrogate and plant-produced proteins. (This is,

unfortunately, standard procedure for testing of most recombinant, plant-produced proteins, such as the insecticidal toxins expressed by Bt crops.) Likewise, the FDA should not rely on "the extent of [sic] structurally and pharmacologically comparable products for which there is clinical experience" in deciding on the extent of pre-clinical testing needed for the PMP, especially if the comparable products in question are extracted from their native [mammalian] host or generated in non-plant systems. On the contrary, due to the many unique aspects of plant expression systems (e.g. unique glycosylation and post-translational modifications) and the near total lack of control over "production conditions" (i.e. rainfall, heat, pest attack, mold infestation, etc., etc.), PMPs should be fully tested as novel drugs in every case, regardless of what "comparators" already exist. In fact, it would seem advisable to subject every batch of PMP derived from plants with different genetic backgrounds or from plants grown in differing environments (including extreme conditions) to a full and stringent set of tests to determine what changes occur in the PMP under varying conditions.

Immunogenicity and allergenicity (964-990)

A growing body of evidence demonstrates puzzling, unpredicted and in some cases dangerous immunogenic responses to biopharmaceuticals produced in engineered cell cultures. Such reactions may lessen or eliminate the drug's potency, induce allergic responses, or even cause auto-immune dysfunction in which the body's natural version of the drug is also inactivated.[15] Engineered drugs that have elicited immune reactions associated with reduced (or loss of) efficacy include the blood-clotting Factor VIII and the multiple sclerosis drug beta-interferon. Auto-immune dysfunction has also been observed. A version of a platelet-inducer known as megakaryocyte growth and development factor (MGDF) produced by Amgen was discontinued in clinical trials because some patients receiving the drug mounted an immune attack on both Amgen's MGDF and their own natural version of MGDF, resulting in bleeding. A similar phenomenon has been observed with several companies' engineered versions of erythropoietin, a top-selling biotech drug that stimulates red blood cell production (most cases involve Johnson & Johnson's Eprex).[16] This adverse effect was caught only after Eprex had been on the market for years.

These immune system responses have taken scientists and regulators alike by surprise. Dr. Burt Adelman, head of research & development at the biotech firm Biogen, found the immune reactions to MGDF "stunning."

> *"The conventional wisdom had been that this was a theoretical risk ... nobody saw it coming. If you're in my business, it's really unnerving."*[17]

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[15] Pollack, A. "Rebellious bodies dim the glow of 'natural' biotech drugs," The New York Times, July 30, 2002.
[16] Tagliabue, J. "Mystery effect in biotech drug puts its maker on defensive, The New York Times, Oct. 2, 2002.
[17] As quoted in: Aoki, N. "Protein therapies spark scrutiny: researchers weigh potential risk of immune responses," The Boston Globe, Nov. 27, 2002.

One problem is that even slight alterations in the processes used to make these drugs in tightly-controlled fermentation tanks can have significant, but difficult-to-detect differences, in the final product. According to the FDA's Chris Joneckis, speaking at a May 2002 FDA workshop:

> *"Despite best efforts to detect product differences and predict the impact of manufacturing changes, these surprises do continue to occur."*[18]

The biggest surprise thus far has involved biotech's flagship product Eprex, which is used to stimulate production of red blood cells to treat anemia. Eprex has been implicated in up to 160 cases of red blood cell aplasia. The aplasia results from disablement of both Eprex and the body's natural version of the substance by the immune system. Despite four years of investigation, it is not known how Eprex induces aplasia in these patients.

Like many biotech drugs, Eprex is generated in mammalian cell culture. Drugs generated in mammalian cells are generally expected to cause fewer such immunogenic reactions because of the similar way in which all mammalian (including human) cells process the proteins they produce. Plants process proteins differently than animals; for instance, they attach different sugar groups to the surface of proteins, which can make even a "human" PMP appear foreign to the immune system, increasing the risks of immunogenic and allergic reactions.

Given the recent surprising incidence of immunogenic reactions to biotech pharmaceuticals, and the increased risks associated with PMPs, it is disappointing to see just four inadequate paragraphs devoted to allergenicity and immunogenicity in the guidance. The FDA should:

1) Develop a robust model for testing PMPs for potential allergenicity and immunogenicity before any more field tests or clinical trials are allowed to proceed, taking special account of the factors that make plant expression systems more likely to generate problematic proteins;

2) Demand that ***all*** PMPs be tested for allergenicity and immunogenicity rather than let applicants "assess the need for allergenicity testing for each product..." Many products might never be tested for allergenicity under this guidance.

3) Require full characterization of all antigenic determinants, especially sequencing of N-glycans, rather than vaguely recommend an evaluation of "the final product for antigenic determinants...," which could be interpreted to mean that the applicant need only report on the presence of such determinants, not elucidate their structure. As adverse reactions to biotech proteins (including PMPs) accumulate, detailed structural data (e.g. precise sequence of glycosyl groups) might help in determining the causes.

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[18] Transcript of "Comparability Studies for Human Plasma-Derived Therapeutics," FDA CBER workshop, May 30, 2002, p. 42.

4) Refer to established protocols for allergenicity testing of novel bioengineered proteins in developing such models for PMPs (in the case of oral biologics, the FAO-WHO 2001 protocol could serve as a model). The FDA completely fails to discuss, mention or even cite any of these protocols, a puzzling omission given the agency's past involvement in promoting development of these testing schemes.

Allergy issues for biopharmaceuticals in whole fruit or vegetable products

(634-36)

The issue of native allergens in biopharm plants and plant products meant for oral use should not be dealt with ***exclusively*** on a case-by-case basis. This is because similar allergenic issues will arise for each crop, regardless of the biopharmaceutical it produces, and it should be possible to establish standardized procedures to ensure the most complete possible removal/inactivation of native allergens. Yet because different transformation events could give rise to unintended effects that in some cases may raise native allergen levels, the standard procedures may have to be amended on a case-by-case basis in light of detailed analyses of the biopharm plant for unintended effects.

Residues of plant-made animal drugs in animal tissues (993-1001)

The guidance devotes just one sentence to the issue of animal drug residues from PMPs in animal food tissues. Rather than refer the matter to the Center for Veterinary Medicine for *ad hoc*, case-by-case consultation, this issue (like so many others raised but not discussed or regulated in this guidance) deserves thorough and systematic consideration. The FDA should also develop strict, detailed regulations for potential animal or human drug residues in animal food products arising from the feeding of biopharm crop materials (e.g. as fodder) or post-extraction byproducts.

Plant-produced pesticide residues in PMPs (951-56)

The FDA advises applicants who wish to stack biopharm crops with biopesticides such as Bt to contact the EPA "regarding the safety of the pesticide." Once again, as with chemical pesticides, it is somehow assumed that the EPA will be in a position to evaluate the novel risks posed by injection, ingestion or dermal application of biopesticide-laced biopharmaceuticals. This is simply not the case. In fact, the EPA has even failed to evaluate the current crop of plant-produced pesticides (the various Bt-derived proteins expressed in corn & cotton) for allergenicity. The EPA's failure to do this means the FDA will have to conduct an independent assessment of the allergenicity and other possible health impacts of Bt endotoxin residues in biologics. Other prospective biopesticides may have other impacts for which testing will be required. For instance, a

USDA scientist has proposed that biopharm crops be stacked with the biopesticide avidin, which not only kills a broad range of insects but also causes Vitamin B (biotin) deficiency in humans and animals that ingest it.

Miscellaneous Comments

Tissue distribution of expression products (398-407)

The guidance requests information on expression levels in various tissues. It should also demand expression levels in these tissues over time throughout the growing season. Companies should also supply detailed data on variability in expression levels (in various tissues) from plant to plant, generation to generation and in plants of the same genetics and generation grown in a wide range of environments, including extreme conditions (e.g. FoE/GEFA 4.6.2).

Environmental review (203-213)

Since APHIS has not conducted an environmental assessment (EA) of a biopharm field trial since 1998, there is little reason to fear "duplication" of environmental reviews among the various agencies. One EA per trial would represent a vast improvement over the current situation. The sentence "APHIS/BRS will identify and evaluate the potential environmental effects posed by field growth of such plants" should be struck, unless the agency demonstrates a serious resolve as well as the necessary personnel and other resources to actually begin doing EAs. And future EAs should not be pro forma, boilerplate exercises, like most of the few prior environmental assessments conducted by APHIS, but rather real studies with real data (see FoE/GEFA 6.5).

Conclusion

Bureaucratic Blinders

The following sentence reveals a fundamental weakness of the guidance document, which in fact is a fatal flaw in all aspects of GE crop "regulation":

> "This document only addresses FDA and USDA guidance; if you have questions regarding the use or safety of pesticides, you should contact EPA." (955-56)

This statement presumes that concerns raised by biopharming break neatly along bureaucratic fault lines. But of course they don't. As discussed above, EPA is competent

to evaluate the risks of oral exposure to pesticides on food, ***not*** the risks of oral, dermal, intramuscular or intravenous doses of pesticide residues delivered together with another bioactive compound (the biopharmaceutical). FDA officials surely know this, but apparently cannot see beyond their bureaucratic blinders. Equally blind is FDA's refusal to concede the obvious need to examine – ***before any such field trials are permitted*** – the potential health effects resulting from biopharm crops contaminating food crops. But FDA refuses to consider this obvious matter not because it isn't worthy of consideration (it most certainly is), but merely because field trials of engineered plants are the USDA's responsibility – bureaucratic blinders once again. A third example is the gaping hole where there should be strict regulatory control of bioengineered plant-made industrial chemicals. EPA should be concerned about environmental impacts, FDA about the potential health impacts (once again) of contaminated food crops, but instead the USDA is left to bungle along (understaffed and unduly influenced by the biotech industry) with next to no regulation of these novel crops. (In fact, industrial chemical crops are even grown under the USDA's weak notification system – 90% of field trial sites for "notifications" are not inspected at all by USDA inspectors.[19])

Economic Self-Interest Ignored...

The biggest failing of our government's "regulatory" system for all genetically engineered crops (including biopharm plants), however, is the failure to give any serious consideration to the economic impacts these crops are having and will continue to have on American farmers, the food industry, and indeed, the very reputation of America as a supplier of safe, healthy food. Government and industry are ***squandering*** this goodwill, this carefully cultivated and earned reputation, every day. Each new contamination episode, each new example of regulatory incompetence, makes government officials, food industry representatives and consumers in foreign nations that import our produce shake their heads in wonderment. "Why does America continue to ignore our elementary demands for safe food?" they wonder. "Why can't Americans seem to enact the most elementary regulations to keep drugs out of the food supply?"[20] Finally, they may decide to import their food from countries whose governments and growers are responsive to their needs. In fact, this process has already begun. Europe has virtually stopped importing corn from the U.S. due to the admixture of inadequately tested GE varieties. Brazil has increased its conventional soybean exports as Britain and other countries turn away from the U.S. market, which consists mainly of engineered soya, in search of non-engineered supplies.

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[19] Personal communication, Dr. James White, APHIS.

[20] The recent ProdiGene episodes in Iowa and Nebraska have reportedly been covered more heavily in European and Asian media than in the U.S.

In Favor of Fanatical "Anti-Regulation" Ideology

The puzzling refusal of the U.S. government to regulate a field posing such patent risks to public health and potential for consumer backlash and massive export losses as drug-producing food crops is explained, at least in part, by a fanatical ideology that holds sway in certain very influential government and industry circles. These "anti-government regulation" fanatics oppose, with truly religious fervor, any government initiative that in any way restricts the scope of private industry to do exactly as it pleases, even when such regulation would prove economically advantageous. Among anti-regulation cultists, government regulators are to be opposed, undermined, hamstrung or co-opted at every turn, in complete disregard of the facts of the case at hand.

Biopharming is an excellent proof of this thesis. Here's the situation in a nutshell:

1) **Contamination assured:** Experts agree that biopharm contamination is inevitable. The promiscuous pollinator corn is by far the favorite biopharm host plant.

2) **Known health risks:** Several of the few PMPs that are known pose demonstrable health risks upon ingestion.

3) **Secrecy fuels legitimate suspicion:** The identities of most PMPs are kept hidden as company trade secrets. Could they pose still greater risks than those that are known?

4) **Government's "GMO force-feeding" policy harms American agriculture:** The U.S. government has thus far pursued a disastrous "force-feeding" policy with respect to GMOs that has resulted in substantial export losses. Bad as it has been, this policy will become still more disastrous in the age of open-air biopharming.

5) **Foreign grain traders adamantly opposed to new GMOs, especially biopharm:** It's not like we haven't been warned. Instead of threatening Europe with WTO challenges, maybe we should start listening to our foreign customers and giving them the products they want – food crops and products that are free of unregulated GMO content.

If one mark of fanaticism is to sacrifice one's rational self-interest for the sake of one's irrational beliefs, then the anti-regulation zealots can truly be said to be under the influence of a deeply irrational fanaticism. Until they are brought to their senses with respect to biopharming, American farmers, food companies and consumers will suffer from their delusions.

MANUFACTURING DRUGS AND CHEMICALS IN CROPS:

Biopharming Poses New Threats to Consumers, Farmers, Food Companies and the Environment

By Bill Freese, Policy Analyst
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for
Genetically Engineered Food Alert

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Manufacturing Drugs and Chemicals in Crops:
Biopharming Poses New Risks to Consumers, Farmers, Food Companies and the Environment

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A note on sources of information:

Report and appendices available online at: www.gefoodalert.org and www.foe.org/biopharm

The USDA's searchable database of field trials of genetically engineered crops, run by Virginia Tech University, can be accessed at www.nbiap.vt.edu/cfdocs/fieldtests1.cfm. Search on phenotypes pharmaceutical protein, novel protein, antibody and industrial enzyme(s) for the crops considered in this report.

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Executive Summary

The biotechnology industry has promised to benefit farmers and consumers with revolutionary new products, yet it has created a host of problems. From contamination of the food supply with StarLink corn, to loss of exports due to commingling and cross-pollination with non-engineered crops, to lawsuits by biotech companies against farmers -- the industry has had negative impacts. Today, a new threat faces us all as a few maverick biotechnology companies are secretly planting a generation of crops that contain biopharmaceuticals, industrial enzymes, antibodies, and even contraceptives.

This report details the threats that these crops pose, the extent to which they have been planted across the U.S., the failure of regulatory agencies to serve the public, and a set of recommendations to protect farmers, consumers, food companies and the environment.

What is "biopharming"?

"Biopharming" is an experimental application of biotechnology in which organisms are genetically engineered to produce pharmaceutical proteins and chemicals they do not produce naturally. While most of these substances are kept secret as confidential business information (6.3), a few known examples include a contraceptive, potent growth hormones, a blood clotter, blood thinners, industrial enzymes, and vaccines. Corn is by far the most popular biopharm plant, followed by soybeans, tobacco and rice. Some 400 biopharm products are reportedly in the pipeline, and over 300 open-air field trials have already been conducted in unidentified locations across the country.

State	No. of Field Trials
Nebraska	37
Hawaii	36
Puerto Rico	35
Wisconsin	27
Iowa	20
Florida	14
Illinois	14
Texas	13
California	11
Maryland	11
Kentucky	10
Indiana	9

Crop	No. of Permits & Acknowledgements
Corn	134
Soybeans	22
Viral-vectored tobacco	10
Rice	9
Tobacco	9

***Table 1** (left): Top twelve open-air biopharm field trial states: 1991 to 6/18/02.*
***Table 2** (above): Top five crops for open-air biopharm experimentation: 1991 to 6/18/02*

Could drugs and chemicals contaminate the food supply?

Contamination of non-engineered or organic corn by engineered insecticides is already widespread. Iowa farmer Laura Krouse has seen her sales of open-pollinated corn drop 50-75% due to genetic pollution with engineered traits. An expert committee of the National Academy of Sciences foresees the same with biopharm crops:

> ***"...it is possible that crops transformed to produce pharmaceutical or other industrial compounds might mate with plantations grown for human consumption, with the unanticipated result of novel chemicals in the human food supply."*** [1]

There is already one report of biopharm contamination. According to Chris Webster of the drug company Pfizer:

> ***"We've seen it on the vaccine side where modified live seeds have wandered off and have appeared in other products."*** [2] (6.3.3)

Biopharm traits could spread through pollen carried by wind or insects, spilled seed, unharvested seed sprouting the next year ("volunteers"), and biopharm seed residues carried by farm equipment to conventional fields (5.4, 7.3.2). The editors of Nature Biotechnology warn bluntly:

> ***"Current gene-containment strategies cannot work reliably in the field ... Can we reasonably expect farmers to [clean] their agricultural equipment meticulously enough to remove all GM seed?"***[3]

Corn is especially risky for pharmaceutical applications because it readily cross-pollinates and its pollen can travel for over a mile. This is demonstrated by engineered StarLink corn, which contaminated food products and corn seed stock with a potentially allergenic protein even *with* the use of gene containment measures. Nevertheless, 2/3 of open-air biopharm field trials have been in corn, and experts warn that current isolation standards will not prevent contamination of normal corn (5.4.1, 6.4.5). Engineered viruses used to infect plants with drug genes could spread to related crops (4.7).

Gene containment mechanisms such as male sterility and tissue-preferred promoters are known to be "leaky." The proposed use of Terminator seed-sterility technology to mitigate biopharm gene flow is unacceptable due to technical flaws, potential health & environmental hazards, and because it would serve to legitimize Terminator's chief intended use, which is to end the practice of seed-saving (5.6). Companies like ProdiGene have also proposed "dual-use" of biopharm plants – extracting the drug/chemical and then selling the rest for use as food or animal feed. Incomplete extraction would mean drug or chemical residues in food products and feed (4.3).

If food becomes contaminated, could these substances harm human health?

* Plants process proteins differently than animals or humans. Thus, experts are concerned that a plant-produced "human" protein could be perceived as foreign by the body and elicit an allergic reaction, including life-threatening anaphylactic shock (4.1).
* Growth factors such as erythropoietin are active at billionths of a gram when injected, and "may be harmful by inhalation, ingestion or skin absorption."[4] Those handling the substance are advised to wear a respirator and chemical-resistant gloves (4.9, 7.7).
* Trichosanthin, a potent abortion-inducing drug, has been introduced into tobacco by means of an engineered virus which is also known to infect tomatoes, peppers, and other tobacco relatives (4.7.3; Appendix 4).
* The research chemical/insecticide avidin causes a vitamin deficiency, and the blood clotter aprotinin can cause pancreatic disease in animals and perhaps humans. Both have been engineered into corn grown out-of-doors (4.5; Appendices 2 & 3).
* Corn-grown industrial enzymes such as trypsin and antitrypsin are known allergens. Trypsin corn is to be grown on hundreds of acres throughout the Corn Belt in 2002 (4.10).

Could plant-grown drugs and chemicals harm the environment?

Conventionally-produced drugs are already a growing pollution nightmare, and plant-grown drugs and chemicals could make things worse (5.1). According to Dr. Glynis Giddings et al:

> ***"Biopharmaceuticals usually elicit responses at low concentrations, and may be toxic at higher ones. Many have physiochemical properties that might cause them to persist in the environment or bioaccumulate in living organisms, possibly damaging non-target organisms..."***[5]

* Aprotinin and other digestion-inhibiting enzymes shorten the lives of honeybees, while avidin is known to kill or chronically impair 26 species of insects (5.3.1).
* The risks to wildlife that eat biopharm corn and other crops increase as scientists learn to generate ever-higher concentrations of drugs and chemicals in these crops (5.1.2, 5.3.2).
* These substances have not been tested for effects on soil life, even though other engineered proteins are known to leak from roots and persist in the soil for months (5.2).

How are plants that grow drugs and chemicals regulated?

The U.S. Dept. of Agriculture (USDA) has primary authority for experimental biopharm crop cultivation. USDA keeps all drug and chemical crop sites secret from the public and neighboring farmers, hides the identity of the drug or chemical in most cases, and condones biopharm companies' preferred practice of "anonymously" planting these crops without identification, security measures or notification of neighbors (6.3). Joe Jilka of ProdiGene, speaking of his company's corn engineered to produce a pig vaccine (TGEV), appears more concerned about theft than public safety (6.3.3):

> ***"...the best way to secure it is to grow it just like any other corn. In other words, the anonymity of it just completely hides it. You know, our TGEV corn grown [sic] was up here by Story City right by the interstate, and no one could have ever seen it."***[6]

USDA's gene confinement measures are intended to "minimize" rather than prevent contamination (6.4.5). The few environmental assessments conducted by the USDA are of poor quality (6.5.2), and show a disturbing willingness to bend the rules. For instance, a trial of alfalfa engineered with industrial enzymes was allowed to proceed despite the presence of non-engineered alfalfa "within 200 yards of the test site," less than the accepted isolation distance. The USDA approved the field trial plan even though it allowed open flowers, increasing the contamination risk, over the objections of the Wisconsin Dept. of Agriculture (6.4.5). USDA is not qualified to evaluate the health risks of biopharm crops (6.4.3), allows commercial use of biopharm plant products (6.4.4), and is too understaffed to exercise adequate on-the-ground oversight, for the most part allowing companies to regulate themselves (6.5). An expert committee of the National Academy of Sciences strongly criticized the USDA for these and other regulatory lapses and deficiencies.

The FDA will play some yet-to-be-defined regulatory role in the later stages of biopharm crop development. When contacted by telephone, FDA representatives were unwilling to speak about the agency's possible involvement in the review of biopharm trials conducted thus far, citing confidentiality claims by business, but public statements are not reassuring:

> ***"And I think to be honest, the FDA is used to applying regulations to manufacturing plants, but not to plants used for manufacturing. So a lot of this is new to us as well, and that's why I won't be able to answer any questions at the end!"***[7]

Would biopharming mean cheaper drugs and chemicals?

Biopharm companies hope that growing drugs and chemicals in plants will be cheaper than conventional production methods through replacement of high-cost production facilities with the flexibility of low-cost contract farmers, meaning higher profits (7.1). However, others believe that biopharming will prove to be expensive and/or non-viable due to difficulties in purifying drugs and chemicals from plants (4.2), the costs of mitigating gene flow (7.3.1, 7.3.2), and litigation and liability costs from contamination (7.4). Barry Holtz of Large Scale Biology, a leading biopharm company, discounts glib predictions of "$5 dollar a gram proteins," estimating that even high-volume plant-grown drugs would cost "hundreds to thousands of dollars a gram" to produce[8] (3.2).

The ***sales*** price would be higher still, as biopharm companies will have to recoup a huge load of sunken costs for research and development of this novel production system. Contrary to industry's oft-repeated promise of cheap drugs and chemicals, one of the only commercialized plant-grown products, the research chemical avidin, actually costs the same as the conventional version extracted from eggs, $46-47 per 5 mg, or over $9,000/gram (3.2). Initial hopes that plants engineered with vaccines could be delivered cheaply in raw form (e.g. bananas) have foundered due to inability to achieve consistent, or sufficiently high, vaccine levels in plants. Some scientists now believe that the vaccines (if successfully developed) would have to be extracted from plants and processed into pill or powder form, increasing the cost of delivery (4.6).

What do drug-growing plants mean for farmers?

Biopharm companies normally contract with selected farmers to grow their drug or chemical crops. But ***all*** farmers are exposed to substantial liability from biopharming, whether they choose to plant these crops or not: 1) Neighboring farmers whose fields become contaminated with drug or chemical traits could sue biopharmers; 2) Biopharm companies could discover their patented drug traits in conventional farmers' contaminated fields, and then sue, alleging violation of the company's "intellectual property" rights; 3) Government agencies could bring enforcement actions for breach of regulations (7.4).

Other disadvantages of biopharming for growers include health risks from inhalation of and contact with potent drugs and chemicals (7.7), intrusive on-site inspections by company managers and government regulators, expensive and time-consuming changes in farming practices (e.g. to mitigate contamination) (7.3), and possible loss of export markets due to contamination (7.5). Against these risks and drawbacks, farmers are being promised a slight premium for biopharm crops, though probably not sufficient to cover the added costs and risks. In 2001, however, ProdiGene-Stauffer Seeds CEO Anthony Laos reneged on the company's promise of a modest 40% premium to corn biopharmers, admitting: "we cannot guarantee acres or premiums."[9] Biopharm acreage is projected by most in the industry to be rather low, so few will plant these crops in any case (7.2).

Are there other ways to produce these drugs and chemicals?

Proven methods include extraction from animal or human tissues and production in animal, bacterial and yeast cell cultures. Newer techniques include plant cell cultures and secretion of biopharm proteins from plant roots into hydroponic media. In contrast to open-air biopharming, these methods are contained, greatly reducing contamination risks; they allow complete control of growth conditions, meaning more consistent drug quality; and purification is easier than from whole-plant tissue. One drug already grown in plant cell culture is the anticancer drug Taxol. Applied Phytologics has experimentally produced the same cystic fibrosis drug (alpha-1-antitrypsin) in both open-air rice plantings and rice cell culture, obtaining very high yields and purity with the latter method. (Appendix 5)

With all these risks, should open-air biopharming be permitted at all?

In a submission to the Canadian Biotechnology Advisory Committee, geneticist and biochemist Dennis R. McCalla and colleagues point to the potential health impacts from inadvertent consumption of plant-grown vaccines, stating that there is a "very high probability" that "plants engineered to produce pharmaceuticals, enzymes [and] industrial chemicals" will contaminate the human food supply. "Only species that are not consumed by humans or by livestock should be permitted for the production of these substances"[10] (3.3; see "Recommendations"). The Genetically Engineered Food Alert Coalition agrees, and recommends that only contained, non-food alternatives to open-air biopharming be allowed.

Endnotes

[1] "Environmental Effects of Transgenic Plants: The Scope and Adequacy of Regulation," Committee on Environmental Impacts Associated with Commercialisation of Transgenic Plants of the National Academy of Sciences. National Academy Press 2002, p 68.

[2] See "Plant-Derived Biologics Meeting" transcript, April 5 & 6, 2000. www fda.gov/cber/minutes/plnt2040600.pdf, p. 77.

[3] Nature Biotechnology (2002). "Going with the flow," Editorial, Vol. 20, No. 6, June 2002, p. 527.

[4] "Erythropoietin: Material Safety Data Sheet," Sigma Chemical Company. Available at www.sigmaaldrich.com.

[5] G. Giddings et al (2000). "Transgenic plants as factories for biopharmaceuticals," Nature Biotechnology, 18, pp. 1154.

[6] "Plant-Derived Biologics Meeting" transcript, www.fda.gov/cber/minutes/plnt2040600.pdf, pp. 77-79.

[7] Michael Brennan of the FDA, as quoted in ref. 5, p. 52.

[8] Plant-Derived Biologics Meeting transcript, www.fda.gov/cber/minutes/plnt1040500.pdf, p. 75.

[9] Stauffer Letter (2001). Letter from Anthony Laos to Customers, Summer 2001, see www.staufferseeds.com.

[10] McCalla et al (2001). "Regulation of Genetically Modified Food: A Submission to the Canadian Biotechnology Advisory Committee," April 17, 2001. See: www.rsc.ca/foodbiotechnology/indexEN.html.

Manufacturing Drugs and Chemicals in Crops:
Biopharming Poses New Risks to Consumers, Farmers, Food Companies and the Environment

Recommendations

1) **Stop granting permits for open-air cultivation of all crops genetically engineered with biopharmaceuticals (such as vaccines), industrial chemicals, or other substances with potential human health impacts.** Crops engineered with industrial biochemicals are not approved for human consumption. Crops engineered with drugs are, at best, approved for consumption only by people with a doctor's prescription.

2) **Allow, at most, the genetic engineering of chemicals or biopharmaceuticals into those non-food crops that do not pose the risk of food contamination.** The USDA has issued "split approval" permits allowing cultivation of ten food crops engineered to produce biopharmaceuticals or chemicals that are not approved for general human consumption in more than 300 field trials conducted across the country from Hawaii, to Iowa, to Florida. The USDA should end this practice to ensure that these substances never enter the human food supply.

3) **Require non-food crops engineered with chemicals or biopharmaceuticals to be cultivated indoors and establish a tracking system governing the handling and disposal of byproducts to prevent environmental contamination.** As there has been virtually no study of the environmental toxicity and persistence of biopharmaceuticals and chemicals engineered into plants, it is irresponsible to permit their open-air cultivation.

4) **Explore contained alternatives to open-air biopharming for production of biopharmaceuticals.** In addition to currently used techniques such as bacterial, yeast and mammalian cell cultures, plant cell cultures and rhizosecretion (secretion of biopharmaceuticals from plant roots) show much promise. Unlike biopharming, these methods are conducted in controlled production facilities, and so do not present the risk of contamination.

1. Introduction

In this report, we will take a close and critical look at an emerging sector of the biotechnology industry known as biopharming, or the production of pharmaceuticals and biochemicals in plants with the techniques of genetic engineering. While still primarily at the research and development stage, there are reportedly 400 plant-grown drugs in the pipeline (Food Traceability 2002), and industry representatives predict an annual market of about $200 billion by 2010 (Guy Cardineau, Biologics Meeting I 2000, p. 23).

1.1 What is a biopharmaceutical?

Biopharmaceuticals are proteins produced by living organisms that have medical or diagnostic uses. While we are accustomed to think of protein as muscle tissue, there are actually about 100,000 different kinds of proteins in the human body, and they perform an amazing array of important functions. As enzymes, they facilitate the reactions that store & release energy and do innumerable other tasks; as hormones, they transmit important signals within the body; as antibodies, they fight infections. Examples of pharm proteins experimentally grown in plants include a topical contraceptive agent, blood thinners, blood clotters, potent growth hormones, and experimental vaccines for animals and humans.

1.2 How are biopharmaceuticals currently produced?

Biopharmaceuticals are traditionally extracted from animal and human tissues (insulin from pig and cow pancreas, blood proteins from donated blood). These substances are also now "grown" in fermentation tanks using engineered bacteria, yeast, plant or mammalian cell cultures. Substances produced with this technique include hepatitis B vaccine (yeast) and erythropoietin, a stimulator of blood cells (mammalian cell culture). Another method involves test-tube "construction" of pharmaceutical proteins from their amino acid building blocks.

1.3 What is "biopharming"?

"Biopharming" is an experimental application of biotechnology that involves manipulating the genetic code of plants to induce them to generate substances they do not produce naturally. While the fermentation methods mentioned above entail genetic manipulation of organisms under strictly controlled conditions, biopharming is normally conducted out-of-doors. This is a crucial distinction, because it is impossible to control all of the many factors that influence the growth, health and propagation of plants in the environment. As we shall see, many of the health and environmental concerns surrounding biopharming arise from the inability to force nature to meet the exacting standards demanded in conventional methods of drug production. Michael Brennan of the Food and Drug Administration (FDA) jokingly admits how much his agency has yet to learn in this regard:

> "And I think to be honest, the FDA is used to applying regulations to manufacturing plants, but not to plants used for manufacturing. So a lot of this is new to us as well, and that's why I won't be able to answer any questions at the end!" (Biologics Meeting II 2000, p. 52)

The "Plant-Derived Biologics Meeting"[1] held in Ames, Iowa in April of 2000 brought together industry representatives, regulatory officials and academics to discuss recent developments and regulatory issues in this field. USDA and FDA are now formulating a guidance document for industry on biopharming that after two years' delay is now due out in May of 2002 (personal communication, Kathryn Stein[2]). A close reading of the meeting transcript confirms Dr. Brennan's observation: many serious and fundamental questions about how to control this technology remain unanswered.

1.4 What is being "pharmed" now?

While still at the research and development stage, biopharming has been out of the laboratory and in American fields for many years in the form of field trials. From 1991 to June 18 2002, 315 open-air field trials have been conducted or approved in the United States. Interest has picked up in recent years, with the majority of trials carried out from 1999 to 2002 (Figure 1). Corn is by far the most popular crop, accounting for over 2/3 of the biopharm plantings (Table 4). Other crops engineered for biopharmaceutical production include soybeans, rice, barley, wheat, canola and tobacco. Biopharm field trials have been conducted on at least 900 acres, probably closer to 1600. The exact figure is not available because the U.S. Department of Agriculture (USDA) fails to report the acreage for many field trials. (See Section 6.2 for tables and additional discussion of this topic.)

1.5 Scope of the analysis

This report will focus on a small subset of the nearly 30,000 field trials of genetically engineered plants that have been conducted in the United States since the late 1980s (Caplan 2001). We have chosen to focus on four categories of particular concern: pharmaceutical proteins, antibodies, novel proteins and industrial enzymes. Some substances with several uses (e.g. pharmaceutical and insecticidal) will also be covered.

2. THE VAST UNKNOWN

2.1 Biopharming as one form of genetic engineering

Like other forms of genetic engineering, biopharming is based on the transfer of genetic material between species that in most cases could never reproduce in nature. A gene providing information on how to build the desired pharmaceutical protein is joined to sequences of DNA from viruses and other organisms to force the plant to generate the foreign protein. If the gene is human or animal in origin, it must often first be manipulated to make it less foreign to the plant. This "genetic construct" is then randomly spliced into the host organism's genome. One common method involves a "gene gun," which literally shoots the foreign genetic construct, which is first coated on tiny pellets of gold or tungsten, into plant cells. Neither the site of insertion in the plant's genome, nor the number of copies of the "transgene" that are incorporated, is controlled. The genetic construct can break apart, resulting in incorporation of

[1] "Biologics" is another term for biopharmaceuticals

[2] Dr. Kathryn Stein was one of the FDA's point persons on biopharm plant regulation. She recently left the FDA to become Vice President of Product Development and Regulatory Affairs at MacroGenics, Inc., of Rockville, MD, an example of the "revolving door" between industry and government regulatory agencies.

gene fragments or failure to incorporate parts of the construct. For instance, Monsanto's MON810 Bt corn, which is planted on millions of acres in the U.S., contains only a fragment of the Bt gene that was supposed to be inserted due to breakage of the genetic construct (Monsanto Corn 1995, pp. 14-15). If the gene (fragment) successfully "takes," the plant cells are then "grown out" to full plants, which produce the pharmaceutical in their tissues, from which it must usually be purified for use.

Another biopharming method employs engineered viruses to infect plants with the drug or chemical gene, forcing the plant to produce the corresponding substance. Tobacco and tobacco mosaic virus are most commonly used in this scheme. The protein is then purified from the plant's leaves or other tissues.

2.2 Unintended Consequences

Unintended effects result from the unpredictable nature of the genetic engineering process, which in turn is conditioned by our still vast ignorance of the ecology of the cell – the subtle and complex interactions between DNA, proteins and various cellular components that constitute the molecular basis of life. A prime example of this ignorance is the recent discovery – based on the results of the Human Genome Project – that human beings have only about 30,000 genes. The prior estimate of 100,000 genes was based on ignorance of the complex DNA-protein interactions by which a single gene is enabled to express many different proteins. Scientists are only now beginning to understand these complex mechanisms (Commoner 2002). Part of the answer must lie in the over 98% of human genetic material that does not code for proteins and which scientists still often refer to as "junk DNA" – "junk" because its functions have not been elucidated.

Internal memoranda from the FDA dating back to 1992, when the regulatory framework for genetically engineered organisms was first established, show clearly that many FDA working scientists had great concerns about the potential of genetic engineering to generate such unintended effects (Alliance for Bio-Integrity). These concerns were never adequately addressed in the final regulations, which were founded on the dubious notion that genetic engineering is merely an extension of conventional plant breeding rather than a radical new technology that poses unique risks (FDA 1992, p. 22991). As a result, the FDA maintains that biotech crops need undergo no mandatory risk assessment process unless they are blatantly different than their non-engineered counterparts.

Time has proven the FDA's more cautious scientists correct. Unintended effects are indeed quite common in genetically engineered crops, and include increased susceptibility to disease, nutritional differences, necrotic lesions, increased lignin content, and reduced levels of aromatic amino acids, to name just a few (see Kuiper et al 2001, p. 516; Benbrook 2001, p. 4; Saxena & Stotzky 2001a). While some of these unexpected effects of genetic manipulation were caught at the development stage, others were revealed only after years of commercial cultivation, for instance, stem-splitting in Monsanto's herbicide-resistant soybeans and increased lignin content in Bt corn. Stem-splitting was triggered by unusually hot conditions not encountered during field trials (Coghlan 1999). This illustrates an important principle: severe environmental stress can trigger unpredictable changes in genetically engineered crops.

It is this combination of unpredictability and ignorance that led Dr. Barry Commoner, one of our nation's most eminent biologists, to conclude:

> "The genetically engineered crops now being grown represent a massive uncontrolled experiment whose outcome is inherently unpredictable. The results could be catastrophic." (Commoner 2002, pp. 46-47)

Biotech crops currently being grown commercially have been engineered mainly for herbicide or insect resistance, traits that are not intended to affect people. If even they pose serious concerns, what is one to think of plant-grown drugs, which are designed to elicit responses in humans and animals?

While there is no foolproof method for detecting unintended effects of genetic engineering, one would at least expect scientists to do everything possible to detect them. In fact, however, much of the basic information required for this task is not being gathered, and newer characterization techniques are not being routinely applied. Kuiper et al (2001) recommend various techniques for determining the DNA and protein sequences of the engineered transgene and its product, as well as characterization of the site of transgene insertion. They also recommend profiling techniques to better detect unpredictable effects. The Codex Alimentarius Commission, which sets international food safety standards, and an expert United Nations panel make similar recommendations (Codex 2002; FAO-WHO 2002). Finally, most plant genomes have yet to be fully characterized, making it difficult to fully explore possible unexpected effects.

3. Why Grow Drugs in Plants?

3.1 Blurring the line between plants and drugs

Since its inception, the enterprise of genetic engineering has followed two different tracks: medical and agricultural. Medical applications, such as the engineering of microbes to produce pharmaceuticals, are conducted under tightly controlled conditions, are normally subject to stringent testing, and sometimes offer clear benefits.[3] In contrast, the cultivation of engineered plants in the open air is inherently uncontrollable because it is subject to the vagaries of nature; for this reason, and because these plants are not adequately tested, they pose largely unexplored risks to human health and the environment.

This likely explains why polls consistently find roughly 90% of the American public in favor of labeling genetically engineered foods (CFS Polls 2002), while attitudes towards medical biotechnology are mostly favorable. Realizing this, biotechnology companies have spent tens of millions of dollars to blur this crucial distinction in the public mind, and cast the light of medical progress on their novel crops. The industry's public relations campaign attempts to convince us that genetically engineered crops are all about saving the poor from hunger, disease and malnutrition, when in fact hardly any resources have thus far been devoted to these worthy ends,

[3] Other more exotic uses – such as gene therapy and germ-line modifications for "designer children" – are of course much more controversial, but have either failed to produce results or remain in the realm of science fiction.

which in any case many believe are better served by other less glamorous, and currently available, means (Altieri & Rosset 1999).

Drug-growing plants, however, are more than public relations. They cross the divide between medicine and agriculture not just in the public mind, but on the ground. Can they deliver on their promise of miracle vaccines and cheap drugs? Much will depend on whether the industry can meet the exceedingly difficult challenge of ***imposing the exacting standards of drug production on the inherently uncontrollable conditions of nature***. As we shall see in Sections 4 and 5, this task involves overcoming a plethora of serious health and environmental obstacles associated with "growing drugs" in the open air. It will also depend on the presumed ability to provide drugs more cheaply by growing them in plants than is possible by other means.

3.2 Will biopharming reduce the cost of drugs?

The main justification for biopharming is the claim that it will reduce the cost of pharmaceuticals. Some industry representatives predict production costs of just "pennies per gram" for some plant-grown compounds, which is supposed to result in lower sales prices as well (Jim Thornton of Demegen, as quoted in Olson 1999). But others project much higher costs, from "hundreds to thousands of dollars a gram" (Barry Holtz of Large Scale Biology Corporation, Biologics Meeting I, p. 75).

The cost of production will of course depend on the particular biopharmaceutical: how difficult it is to grow and purify, what containment measures are needed, the expense of testing for health and environmental impacts, its market value, and other factors. Despite these widely varying cost projections, everyone seems convinced that a plant-produced drug would always be cheaper than its conventionally produced counterpart. But is even this true? Let's look at a concrete example.

Avidin, which will be discussed further in Section 4.5.1, is biopharm's price-busting poster child. Everyone agrees that the corn-produced version is many times cheaper to produce than avidin obtained in the conventional manner from eggs (Hood et al 1997, pp. 304-05; USDA Avidin 2000; Olson 1999); one report suggests production costs of only $50/gram, 1/20th that of egg-avidin ($1,000/gram) (Seed and Crops Digest 1998). Yet from February to May 2002, the selling price of corn-derived avidin from its only supplier, Sigma Chemical Company, has ranged from roughly the same to 200% more than that of the same company's egg-derived avidin. In addition, ***corn-derived avidin sells for 16 to 18 times its reported production cost*** – quite a hefty profit margin, to say the least. And when one considers that farmers producing avidin corn are only being paid less than 1 1/2 times the price offered for normal corn, it becomes clear that neither consumers nor farmers benefit from plant production of this protein.

While one cannot extrapolate from just a single example, the case of avidin corn at least sounds a note of caution in the loud chorus of industry voices telling us that biopharming will reduce the cost of drugs.

3.3 Dissenting Voices

Some well-respected figures in the world of genetics have their reservations about biopharming. William Haseltine, chief executive of Human Genome Sciences Inc., worries that plants may adversely modify the drug protein, for instance cleaving it or folding it incorrectly:

> "We believe there are enough risks in the development of new drugs. To add another one – that is, the method of production – is unwise" (as quoted in L.A. Times 2001).

At least one company, Britain's Axis Genetics, which had brought certain plant-grown drugs to the stage of clinical trials, went out of business in early 2000, unable to raise capital (Olson 1999; NYT 2000). Large Scale Biology Corporation, formerly Biosource Technologies, conducted the first field trial of a plant-grown pharmaceutical in 1991, but still does not have a single drug in clinical trials (NYT 2000).

Health concerns associated with growing drugs in an alien organism under uncontrolled conditions provide additional grounds for questioning this technology.

In a submission to the Canadian Biotechnology Advisory Committee, geneticist and biochemist Dennis R. McCalla and colleagues point to the potential health impacts from inadvertent consumption of plant-grown vaccines. They state that there is a "very high probability" that "plants engineered to produce pharmaceuticals, enzymes [and] industrial chemicals" will contaminate the human food supply. "Only species that are not consumed by humans or by livestock should be permitted for the production of these substances" (McCalla et al 2001).

4. Health Risks of Drug-Growing Plants

4.1 Challenging the Immune System

Some of the substances being grown experimentally in plants are designed to elicit immune system responses – in particular oral vaccines. Others may stimulate the immune system unintentionally. In either case, the potential impacts of plant-grown, immunoactive compounds on human health require careful consideration.

4.1.1 Genetically engineered foods pose risk of allergies

Development of allergies – which comprise one class of immune system dysfunction – has long been one of the chief concerns surrounding the introduction of new genetically engineered foods (ILSI 1996, FAO-WHO 1996, 2000, 2001). Allergies commonly develop upon exposure to so-called "novel" proteins (to which people have never or only rarely been exposed), and biotech crops often produce such novel proteins. For instance, the bacterial-derived insecticidal proteins in some varieties of engineered Bt corn are suspected allergens (Bernstein et al 1999; SAP Bt Plant Pesticides 2001).

Allergies are often taken lightly, especially when weighed against the potential benefits of a new drug. Yet they affect a large number of people (about 2.5% of American adults and 6-8% of children, or 8 million in the U.S.), and the incidence of allergies has been rising in recent years

for unknown reasons (SAP StarLink 2000b, p. 11; Wal 1998, p. 413). Reactions are not limited to watery eyes; some experience intense itching and welts, others life-threatening anaphylactic shock, which kills an estimated 150 Americans each year. Those who know they have severe allergies can get prescriptions for Benadryl or similar drugs, which they self-inject at the onset of a reaction. Otherwise the estimated 29,000 episodes of anaphylactic shock in the U.S. each year (Bock et al 2001) would probably result in many more fatalities. If a plant-grown drug with allergenic properties were to contaminate the food supply, unsuspecting individuals prone to allergies would be unable to take any action to avoid consuming the contaminated food, with potentially fatal consequences.

Allergies and allergic reactions can be induced by inhaling the allergen, consuming it, or even by skin contact. While concern has focused mainly on food allergens, Britain's Royal Society recently urged that biotech crop assessments also consider inhalant and dermal exposure. Since infants, young children and farm/food industry workers are especially susceptible, the Royal Society also recommended some form of surveillance for these groups to monitor for possible reactions upon introduction of a new biotech crop (UK Royal Society 2002).

4.1.2 Glycosylation

One of the major concerns with plant-grown drugs is that they often have slightly different structures than their natural counterparts produced in humans or animals. This is because plants and animals often attach different types of carbohydrate groups to proteins (forming glycoproteins) in a process known as glycosylation. The different glycosylation pattern of plants can adversely affect the efficacy of the plant-grown drug (Matsumoto et al 1995), and also elicit unwanted immune system responses, including allergic reactions (Dr. Gary A. Bannon, as quoted in NYT 2000).

Unfortunately, it appears that some researchers are not yet testing their experimental plant-grown drugs for possible allergenicity (Biologics Meeting I 2000, p. 104; II, p. 28). Especially troubling is the fact that the FDA does not even have a model in mind for testing the allergenicity of plant-grown drugs (Ibid II, p. 56).

4.2 Contamination, Purification and Degradation

Another concern with plant-grown drugs is how to extract them from the plant's tissues. They must be separated from thousands of other plant constituents, some of which may be toxic or allergenic, as well as any applied chemicals. The drug must be obtained in pure form, which can necessitate harsh purification procedures that risk damaging its structure. Improper folding or cleaving of the protein can also impair its efficacy and safety. If the drug is to be stored in seeds or other plant tissues until needed, measures must be taken to ensure that it does not break down or suffer loss of activity.

4.2.1 Natural toxins and allergens

Tobacco is one of the favorite crops of biopharm researchers. It is composed of 4,000 compounds (Repetto 1990) that would need to be excluded in purifying a tobacco-grown drug. Such purification would require the removal of nicotine, of course, and other glycoalkaloids

natural to tobacco. This can be a difficult proposition, and even interfere with the testing of prospective drugs (Mor et al 1998, p. 450). In the case of a soybean-grown drug, the purification protocol would have to contend with bioactive and/or allergenic substances such as protease inhibitors and lectins. Elimination of allergens is an especially ticklish proposition in view of reminders from allergy experts that most plant allergens have yet to be identified and characterized (SAP MT 2000, p. 21).

Purification would have to be especially stringent for drugs intended for intravenous or other non-oral use, because the risk of severe allergic or other reactions increases greatly when a drug is able to skip past the body's natural mucous and digestive barriers and gain direct access to the bloodstream.

4.2.2 Plant viruses

Plant viruses must be counted a great unknown. They are said to be fairly common on many crops, and can sometimes exist without causing severe or even noticeable symptoms of plant disease (Biologics Meeting I 2000, p. 44). According to virus expert Dr. Allen Miller, researchers who work with plant viruses sometimes test positive for antibodies to viral proteins (Ibid I, p. 44). Since allergies involve the formation of a particular type of antibody (IgE), it is conceivable that injection of a virus-contaminated drug directly into the bloodstream could be very dangerous, provoking severe allergic reactions.

Thus, it is disappointing to learn that at least one company does not plan to specifically test for or eliminate all plant viruses in its purification procedures, as evidenced in this interchange between Gordon Moore of Centocor and Douglas Russell of Integrated Protein Technologies, a division of Monsanto:

> GM: Would you take the position that it will not be necessary to incorporate purification steps designed to remove virus?
>
> DR: I think the column techniques we're using have been proven to remove some of those factors, but it isn't where we target our process validation. We'd be more thinking about some of the factors of the endotoxins that may be – or protein components that may be particular to plants different from mammalian. We'd put our focus where it's really needed to study." (Ibid I, p. 43)

Asked about government regulations concerning removal of plant viruses, Kathryn Stein of the FDA replied:

> "I think that we would not ask for validation studies to show that these viruses could be removed if the process is a robust purification process and we have no concerns about possible infectivity in humans." (Ibid II, p. 74)

Infectivity, of course, is not required for allergic reactions. In fact, because we still haven't classified the vast majority of viruses, much less elucidated their structures (Dr. Charles Rupprecht of the Centers for Disease Control, Ibid I, p. 126), it might be impossible to test for and eliminate many viruses.

4.2.3 Pesticides and other contaminants

Plants growing drugs may require more protection and care than normal food and feed crops. A crop infested with insects or fungi may be judged unsuitable as a source of the drug or give reduced yields, spelling financial hardship for the grower, especially if the plants represent a significant source of income. Therefore, biopharming may well generate pressure for increased use of pesticides, which raises another set of safety concerns.

Michael Brennan of the FDA asks whether "the use of pesticides [will] be restricted at a certain time before harvest," and also raises troubling issues "like heavy metals and sewage treatment and fermentation..." (Ibid II, p. 53), referring to the common practice of treating agricultural fields with municipal sewage sludge, which contains uncharacterized toxins, pharmaceuticals and their degradation products as well as pathogen-containing human fecal matter. While Brennan insists that "filter sterilization of a final product is not going to be a remedy for excessive bioburden [i.e. contamination] during production," (Ibid II, p. 51), it is unclear how the understaffed FDA will find the resources to monitor and enforce any restrictions on pesticide use or other contaminants in the field.

One partial "solution" to the chemical pesticide issue would probably involve "stacking" the biopharm crop with gene(s) for bioinsecticides, such as avidin (see Section 4.5) or the Bt toxins presently engineered into Bt corn and cotton. Bt toxins present unanswered questions concerning allergenicity[4] (SAP Bt Plant Pesticides 2001, p. 76) that U.S. regulatory agencies have thus far largely ignored (Freese 2001b), while avidin elicits immune system responses and causes B vitamin deficiency. Failure to completely remove co-engineered insecticides from plant-grown drugs could thus pose health risks, especially with injected drugs. In addition, experts recommend the application of more sophisticated techniques (e.g. profiling) to increase the likelihood of detecting the unintended effects anticipated with such "stacked" crops (Kuiper et al 2001, p. 523).

4.2.4 Protection over time – storage and degradation

The main force driving the use of plants as vehicles to grow drugs is the anticipated cost savings over other production systems. One cost-cutting aspect of drug-growing plants is the presumed ability of seeds to serve as inexpensive "warehouses" for the drug (ProdiGene Benefits 1999). Storing the drug in seeds versus more expensive storage requirements with other systems (e.g. refrigeration) could offer substantial cost savings to the company, provided that the seed is as durable and protective a storage system as is hoped.

One important factor in this regard will be to protect drug-bearing seeds from storage grain pests and molds. Once again, broad-spectrum pesticides and fungicides engineered into the crop (e.g. avidin) will probably be the preferred method, especially given the likely need to avoid the use of synthetic chemical agents.

Another factor to consider is degradation of the drug in the seed over time under the broadest possible range of storage conditions, such as high temperatures and humidity. If seeds or other

[4] The EPA's Scientific Advisory Panel (SAP) concluded that **"Bt proteins could act as antigenic and allergenic sources."**

drug-bearing parts of crops require special storage conditions, costs go up, eroding the competitive edge of this production system.

4.3 Dual Use

Another approach to reduce costs is to first extract the biochemical, and then sell the remaining crop residue into the feed or food chain. This tactic is proposed as one way to defray the high costs of 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. The costs of isolating human serum albumin from starch potatoes, for example, could be largely covered by concomitant starch production." (G. Giddings et al 2000, p. 1151)

At least one leading biopharm company that specializes in corn is also thinking along these lines: "Byproduct credits from oil or meal sales can lower production costs" (ProdiGene Protein Products 1999). The economic pressure for dual use of this sort will increase with the acreage planted, which in turn depends on the demand for the particular biopharmaceutical. Companies like Monsanto's Integrated Protein Technologies and Epicyte plan to grow substances such as monoclonal antibodies (NYT 2000) and contraceptive corn (Section 4.8) on thousands or tens of thousands of acres to meet anticipated demand. Without dual use, what would be done with the huge quantities of by-products? To give an idea of the magnitude of the problem, 1,000 acres of corn yields over 8 million pounds of corn kernels alone, not counting other parts of the plant[5].

The prospect of dual use raises troubling questions for both animal and human health. In the case of corn, companies appear most interested in extracting the biopharmaceutical only from seed, even though other parts of the crop (e.g. cornstalks and leaves) will also likely contain some level of the biopharm protein. The effects of drug-containing cornstalk fodder on animals must be carefully considered, especially since such fodder might represent a large portion of the animal's diet. Secondly, corn kernels from which the biopharmaceutical has been extracted will likely contain residues of the drug, and of any pesticides engineered into the plant to protect it in the field and in storage. Will it be cost-effective for the company to ensure complete removal of all contaminants from food and feed "by-products"? Should consumers accept the additional level of risk this would pose to food safety?

4.4 The Dose Makes the Poison

The Holy Grail of biopharm researchers is to achieve "high expression levels" of the desired foreign protein – in other words, high concentrations in plant tissue (ProdiGene Protein Products 1999). Yield and hence profit depend directly on the amount of the biochemical that can be generated by and extracted from the plant. Expression levels of engineered biochemicals are usually reported as a percentage of the "total soluble protein" (TSP) produced by the plant. For instance, Bt insecticidal proteins in commercialized lines of corn are expressed at roughly 0.1 – 0.3% of TSP in kernels, meaning that $1/1000^{th}$ to $3/1000^{th}$ of the seed's total water-soluble (i.e. extractable) protein consists of the Bt protein (Kota et al 1999, p. 1840). Even this low level has

[5] Conservatively estimating 150 bushels/acre; 1 bushel = 56 lbs.

raised allergenicity concerns among experts (SAP Bt Plant-Pesticides 2001, p. 76), and most drug-growing applications will require much higher expression levels to be economically viable.

The concentrations of engineered drugs and biochemicals in plants have risen dramatically in recent years as genetic engineers learn the tricks of the trade. While early experiments generated levels on the order of 0.001% to 0.02% of the plant's TSP (G. Giddings et al 2000, p. 1152), 100 to 1,000-fold higher levels are common today. For instance, the blood clotter aprotinin is produced in corn at up to 0.43% of TSP, avidin at 3% (Zhong et al 1999, p. 354). The protein-degrading enzyme trypsin has been generated transiently at the astounding level of 19% of TSP in corn (ProdiGene Protease Patent 2000). While the threshold expression level for economic viability will vary depending on the value of the particular drug, the overall trend is clear – more is better...

Better, at least, for profitability, but not necessarily for human health or the environment. If "the dose makes the poison," then inadvertent exposure to some drug-producing plants that pose lesser or no risk at low expression levels may become problematic as concentrations rise. In terms of regulation, a biopharm crop approved on the basis of a low expression level may be subsequently engineered for higher-level expression without adequate review of the potential impacts. As an EPA official told the author when presented with evidence of the potential allergenicity of Bt crops: "Once a crop is commercialized, it can be very difficult to recall." For at least some crop-grown biochemicals, then, a difficult tension will arise between industry's drive to maximize profit and protection of human health and the environment.

4.5 Plant-Grown Chemicals with Insecticidal Properties

Several of the drugs and chemicals being engineered into crops are extremely versatile compounds, with applications in biological research, medicine and agriculture.

4.5.1 Avidin-producing corn

Avidin corn was developed jointly by ProdiGene, Pioneer Hi-Bred International and the U.S. Department of Agriculture (USDA) by engineering the gene for chicken egg avidin into corn (Kramer 2000; Hood et al 1997, p. 292). Avidin corn has been grown in field trials since 1993 (NAS 2002, p. 181), and corn-derived avidin supplied by ProdiGene is presently sold as a research chemical by Sigma Chemical Company.[6] Because avidin kills many stored-grain insect pests, avidin corn is also proposed for use as food and feed corn, and as an "insect-resistant background host plant germplasm" for production of "other valuable bio-pharmaceutical or industrial proteins" (Kramer 2000).

Avidin impairs the immune system, reproduction and prenatal development

Avidin is found naturally in the egg white of bird, reptile and amphibian eggs. It deactivates biotin, an essential B vitamin, and can cause biotin deficiency. Experiments on mice (Baez-Saldana et al 1998, p. 431), rats (Kumar & Axelrod 1978; Rabin 1983) and guinea pigs (Petrelli et al 1981) have shown that biotin deficiency weakens the immune system. In hamsters, biotin deficiency can also impair reproductive function and prenatal development (Watanabe 1993). In

[6] Product number A8706; see www.sigmaaldrich.com.

humans, consumption of large quantities of avidin in the form of raw egg whites is known to cause dermatological, neurologic, and ocular disorders (Dorland's Medical Dictionary 1994). Biotin deficiency is also thought to have adverse effects on the human immune system (Baez-Saldana et al 1998, p. 435).

Has avidin corn already entered the food supply?

Informed sources who wished to remain anonymous detailed several instances in which ProdiGene and farmers it contracted to grow avidin corn failed to follow gene containment protocols designed to prevent escape of the avidin gene. These lapses included failure to "detassel" (cut off the pollen-producing tassel of the corn plant) and failure to clean farm equipment after harvesting avidin corn (necessary to prevent spread of avidin corn seed to conventional fields). Avidin corn is supposed to have sterile pollen, which is touted as a biological containment measure that prevents escape of the avidin gene (NAS 2002, p. 181). However, 15% of supposedly "male sterile" avidin corn plants in fact have "limited fertility," while 3% are fully fertile (see Section 5.6.2). Thus, contamination of food-grade corn with avidin could have already occurred due to growth of avidin "volunteers" in conventional fields, or through cross-pollination. Though ProdiGene and USDA personnel have claimed in conversation that avidin corn is presently being grown on just five acres (Ibid, p. 181), there are no publicly available documents to confirm or refute this claim.

The dose makes the poison?

Does avidin corn contain enough avidin to be hazardous to human health in the event it contaminates the food supply? Kramer (2000) reports that humans can suffer avidin-caused "egg white injury" from consuming "a couple dozen raw eggs a day for several months." Based on figures provided by Hood et al (1997), 100 grams of avidin corn (less than ¼ lb.) contains as much avidin as 14 to 27 large eggs (p. 304).[7] In addition, experiments show that only 18% of the total avidin activity is lost during dry milling (Ibid, p. 297). Dry milling is the process used to prepare corn flour, corn grits and similar products from whole corn. Dr. Kramer admits that: "Long-term ingestion of high levels of avidin maize may be a problem, because a biotin deficiency can decrease the growth rate of mice and affect reproduction." Of course, solutions are always available:

> "...avidin has an antidote (biotin), which can be used to prevent toxicity or to rescue potential victims from adverse effects. Food and feed uses of avidin maize might involve processing that includes supplementation with the vitamin" (Kramer et al 2000, p. 672).

It is probably safe to assume that most "potential victims" would prefer that the toxin be kept out of the food supply in the first place rather than depend on the food processing industry to "rescue" them from adverse effects through adding an "antidote."

Unintended consequences

Such a "solution" to an engineered problem would not be justified even if avidin corn were otherwise a thoroughly safe and well-characterized crop. In fact, however, scientists have detected a number of unexpected – and yet unexplained – effects of engineering the avidin gene

[7] Avidin corn kernels contain 150-300 mg avidin per kilogram of seed, depending on the growing location. One large egg (50 grams) contains 1.1 mg avidin. See Appendix 2 for a more detailed treatment of avidin corn.

into corn. Besides male sterility, these include greatly varying levels of avidin in individual corn kernels (Kramer et al 2000, p. 670), two-fold difference in avidin content of avidin corn populations from different generations and growing locations (Hood et al 1997, p. 304), loss of the herbicide-resistance trait (Ibid, p. 298), and in some cases female sterility and other toxic effects (Ibid, p. 304).

Could corn-derived avidin be allergenic?
Natural avidin from egg-white is known to cause immune responses in humans (Subramanian & Adiga 1997, Meyer et al 2001), though it apparently has no history of causing allergies (Langeland 1983). What about corn-grown avidin? Hood et (1997) determined that corn-avidin has a different glycosylation pattern than egg-white avidin, but failed to fully characterize the difference (pp. 302-03). As discussed in Section 4.1.2, plant glycosylation patterns raise the risk of allergy.

Avidin to be engineered into corn together with pharmaceuticals?
Despite the known risks and unexplained effects discussed above, there is already talk of stacking avidin corn with other bioactive substances (Kramer 2000), compounding one poorly characterized genetic experiment with a series of others. It is hoped that the presence of avidin would protect the corn grain and its co-engineered biopharm protein(s) against pest infestation and degradation, providing by far the cheapest storage option for the engineered protein – grain silos (ProdiGene Benefits 1999).

Avidin corn: industry and government neglect public safety
The USDA jointly developed avidin corn with ProdiGene, and has promoted this crop in its popular literature (USDA Avidin 2000, an article entitled "Avidin: An Egg-Citing Insecticidal Protein in Corn"). Thus, it is not surprising that the agency overlooked both ProdiGene's sloppy genetic containment practices and the partial nature of avidin corn's "male sterility." Given the likelihood of contamination and the proven and potential adverse impacts of avidin, avidin corn should no longer be grown in the open air. See Appendix 2 for a fuller discussion of avidin corn.

4.5.2 Aprotinin and other protease inhibitors

Aprotinin corn was developed by scientists with ProdiGene, Pioneer Hi-Bred International, Eli Lilly & Company and PE-Applied Biosystems by inserting a modified gene sequence for cow aprotinin into corn (Zhong et al 1999).

Medical uses of aprotinin
Aprotinin is a protease inhibitor – a substance that inhibits the action of protein-degrading enzymes. A version of aprotinin derived from cow lung tissue is sold by Bayer under the name of Trasylol, which is used as a clotting agent to reduce blood loss in heart surgery (Landis et al 2001) and in the treatment of acute pancreatitis (Belorgey et al 1996, p. 555). In rare first-use cases, aprotinin has caused life-threatening anaphylactic reactions, a risk that increases significantly (up to 5% of cases) upon re-exposure (Trasylol Label 1999). Since aprotinin is infused intravenously for these medical applications, it may not pose the same risks when ingested, inhaled or through skin contact, though studies of these latter routes of exposure appear to be lacking.

Has the food supply been contaminated with aprotinin?

Aprotinin corn has been grown at least since 1998, when it was reportedly cultivated by farmers under contract with ProdiGene's partner, Stauffer Seeds, in Hamilton County, Nebraska (Seed and Crops Digest 1998). However, the USDA biotech website does not identify a field trial of aprotinin corn until 2002 in Hawaii (Permit No. 01-187-01r). Friends of the Earth has been unable to obtain further information about field trials of aprotinin corn from either the USDA or FDA, so it is difficult to judge the potential for contamination of food-grade corn. However, two factors give cause for concern: 1) ProdiGene's history of sloppy gene containment with avidin corn; and 2) Aprotinin corn's pollen is apparently fully fertile,[8] heightening concerns about cross-pollination with normal corn.

Allergenic potential

Aprotinin is a fairly stable molecule, resistant to degradation by enzymes, acids and heat (Sigma Aprotinin), all common characteristics of food allergens (SAP Bt Plant Pesticides 2000, p. 26; Sampson, H. 1999). Aprotinin has apparently not been specifically tested for plant glycosylation patterns, whose presence would also raises allergy concerns (Zhong et al 1999, p. 353). As noted above, aprotinin i.v. has been found to cause anaphylaxis, a life-threatening allergic reaction.

Pancreatic disease from ingestion of protease inhibitors

Animals fed protease inhibitors exhibit retarded growth due to interference with the digestive activity of enzymes like trypsin that are secreted by the pancreas. This inhibitory effect on trypsin causes the pancreas to compensate by secreting more trypsin, leading to abnormal enlargement and cell proliferation of the pancreas. Prolonged feeding of soybean trypsin inhibitors has been shown to cause pancreatic cancer (SAP MT 2000, pp. 31-33). There is evidence that aprotinin (Dlugosz et al 1988) and other protease inhibitors (SAP MT 2000, p. 31) stimulate oversecretion of trypsin and other digestive enzymes in humans as well as animals. According to expert advisers to the EPA: "This would indicate that the human pancreas at least responds in a negative fashion to the effects of a protease inhibitor" (Ibid, p. 31). EPA's experts therefore recommend that transgenic plants expressing protease inhibitors such as aprotinin be subjected to animal feeding experiments before approval. Protease inhibitors may also have other toxic effects, such as depletion of essential amino acids (Kleter et al 2000, section 2.2.3).

Attempts to discover whether the USDA or FDA had conducted any tests to gauge the potential health risks posed by open-air cultivation of aprotinin corn were unsuccessful. The USDA's limited response to a Friends of the Earth Freedom of Information Act request (6.3.2) contained nothing concerning aprotinin. An FDA scientist said that while the FDA might consult with the USDA on certain biopharm plantings, she was not at liberty to discuss aprotinin or any particular product (personal communication, 2/8/02, Kathryn Stein, formerly of the FDA).

See Appendix 3 for a fuller discussion of the health and environmental risks of aprotinin and its production in corn.

[8] There is no indication that aprotinin corn is even partially male sterile in either of the two major documents on aprotinin corn – Zhong et al (1999) and Aprotinin Patent (1998).

4.5.3 Gene stacking and synergistic effects

Biotech companies are currently experimenting with "gene stacking," or splicing combinations of pharmaceutical and/or insecticidal genes into crops (Kleter et al 2000, 2.2.4). Combinations of drugs/insecticides can have unexpectedly strong (i.e. synergistic) effects, as ProdiGene found in tests conducted on aprotinin and wheat germ agglutinin (Aprotinin Patent 1998). This particular combination was found to be extremely toxic to insects, but since these two classes of substances (protease inhibitors and lectins) appear to have similar mechanisms of toxicity in insects and mammals, stacking of these genes might have human health impacts as well.

4.6 Growing Vaccines in Plants

One of the most highly touted potential applications of biopharming is the edible vaccine. By growing and storing vaccines in plants, scientists hope to reduce the cost and ease distribution of these valuable drugs so they can reach the people who need them most. Potential advantages of these prospective food-drug hybrids include elimination of the use of needles, lower storage costs and ease of transport to remote villages (Dr. Jose Luis DiFabio, Biologics Meeting I 2000, p. 10).

Edible vaccines against "traveler's diarrhea," hepatitis B and rabies have reached the stage of clinical trials. Tomatoes, potatoes and tobacco (purification required) are the favored host plants (Biologics Meeting I 2000, pp. 80-100). Although trials have yielded some hopeful results, how feasible is it to grow safe and effective vaccines in plants?

4.6.1 Determining the correct dose

Because oral vaccines must contend with the harsh and variable environment of the gut, which changes over time and varies from person to person, determining the correct oral dose is difficult. Digestive enzymes and stomach acids will degrade the vaccine unless it is protected, which by some estimates will necessitate use of anywhere from 10-100 (Dr. Liz Richter, Biologics Meeting I 2000, p. 93) to 1,000-10,000 (John Howard of ProdiGene, as quoted in L.A. Times 2001) times as much edible vaccine as would be needed for injection. The stomach's variable acidity and other factors influence how much vaccine survives digestion to "get through" to the immune system. A dosage that is correct for one person at one time may well be too little or too much for another. Thus, selecting the "correct" dosage for a standardized oral vaccine would seem to be an extremely difficult proposition.

4.6.2 Dosage problems will necessitate processing

These generic problems of oral administration are compounded by other dosage uncertainties in the case of edible vaccines. For instance, scientists have not succeeded in obtaining consistent levels of vaccine in various generations or individuals of the same plant line, even when grown under identical greenhouse-controlled conditions (Liz Richter, Biologics Meeting I 2000, p. 96). Other factors that can influence vaccine levels in transgenic plants include degree of ripening, plant health, length of growing season, weather conditions, light levels, pest infestation and genetic background of the plants -- all of which obviously become more important if vaccine plants are to be grown out of doors rather than under strictly controlled greenhouse conditions.

Edible vaccines not to be eaten as raw fruits or vegetables

These unexplained differences in vaccine levels from plant to plant, as well as size differences between individual fruit, make it impossible to "prescribe" a vaccine fruit or vegetable in raw form (Ibid I, pp. 95-96; II, p. 91). ***According to Hugh Mason, a leading researcher in the field of edible vaccines, "now we believe that processing will be completely necessary and we don't really contemplate delivering individual fruits."*** He suggests that processed versions of food vaccines could take the form of freeze-dried powders or perhaps pills (Ibid II, pp. 88-89). Batch processing of some sort would presumably make it possible to level out plant-to-plant, line-to-line and generational differences in vaccine levels, though this would also require strict testing and monitoring of vaccine levels in each batch. Processing and quality control would inevitably increase the costs of production and distribution. This is a significant issue for the very feasibility of this technology vis-à-vis other vaccine production systems, since one of the strongest arguments in favor of edible vaccines has always been the presumed low cost and ease of distribution.

It is very important that these limitations to development of edible vaccines be openly and honestly discussed, since the media have already latched onto the dangerously simplistic notion that poor 3rd World children might be vaccinated by simply eating a banana or other fruit (Newsweek International 2002).

4.6.3 Immunity or tolerance?

A second problem with edible vaccines is the poorly understood phenomenon of "oral tolerance," the natural process by which the immune system learns to accept or "tolerate" an ingested protein rather than respond to it via formation of antibodies. While the body naturally develops tolerance to food proteins (food allergies occur when the immune system fails to do this), oral tolerance is undesirable in the case of edible vaccines, because the vaccine must elicit an immune system response in order to be effective. Experts view this as "a very serious handicap" of oral immunization (Maekelae 2000, p. 17). Experiments with potatoes engineered to produce a vaccine against traveler's diarrhea caused by Norwalk virus indicated development of partial oral tolerance in mice (Mor et al 1998, p. 451). This continues to be an issue of great concern (Biologics Meeting II 2000, p. 28) because it raises the prospect that edible vaccines could actually weaken rather than strengthen protection against the disease agent.

Since oral tolerance seems to occur more frequently with soluble antigens, the main strategy being developed to avoid this problem involves assembling the components of the vaccine into insoluble particulate form to more closely resemble the live virus (Mor et al 1998, p. 450). The efficacy and perhaps the safety of an edible vaccine would then seem to depend on preventing solubilization of the antigen and maintaining its desired particulate formation (e.g. virus-like particles) in the food product. Here, stability over time – at various degrees of ripeness, under differing environmental conditions of growth and storage – are key issues (Biologics Meeting II 2000, p. 54).

4.6.4 Acceptance of the "delivery system"

Unfortunately, many of the fruits and vegetables that are more attractive as foods (e.g. bananas, apples, tomatoes) naturally produce low levels of protein, and so might be difficult to engineer to

produce adequate levels of vaccine (Mor et al 1998, p. 453). This is particularly a problem when one recalls that much larger amounts of vaccine are required for oral immunization than for injection.

4.6.5 Other potential health risks of edible vaccines

Some of the issues raised above with respect to the efficacy and viability of edible vaccines – dosage control and consistency, oral tolerance and stability – also present health concerns, as we have seen. Additional potential health risks include allergic reactions from plant glycosylation patterns (see Section 4.1.2) and unintended effects (Section 2.2). Some transgenic tomatoes engineered by Dr. Hugh Mason with an experimental vaccine against traveler's diarrhea have crinkled leaves, an unintended effect of the vaccine engineering process that he was unable to explain. Mason speculates that it may be due to "an excess number of chromosomes," but offers no evidence to support this explanation (Newsweek International 2002). Experiments with mice indicate that ingestion of the vaccine induces an immune response, and human clinical trials are planned for sometime in 2002. But shouldn't such an unintended effect be satisfactorily explained before human trials are initiated? It also raises questions about other, potentially more dangerous, unintended effects that may not be so easy to detect as crinkled leaves.

4.6.6 Plant-produced vaccines versus other production systems

In a comprehensive review of the history and current developments in the field of vaccines, P. Helena Maekelae of the Finnish National Public Health Institute is excited about new developments in vaccine production, but pessimistic about the prospects for edible vaccines:

> "The idea to produce vaccine antigens as protein components of edible plants and use these as oral vaccines sounds attractive: the production in transgenic plants is indeed feasible and immune responses have been obtained in mice fed the plant. However, ***it is hard to believe in it as a realistic goal just because in vaccination, much more than the antigen needs to be controlled.*** It is not justified by the ease of mass production either since the amounts of protein antigen in a vaccine dose are relatively small." (Maekelae 2000, p. 17, emphasis added).

4.7 Plant Viruses: Growing Drugs in Diseased Plants

Another rather bizarre way to grow drugs in plants involves infecting them with genetically engineered viruses. The desired gene is first inserted into the virus, which is then used as a vector (i.e. carrier) to infect the plant with the foreign gene. The plant is thus forced to produce the foreign substance along with the virus's own proteins, in the process becoming diseased. The target protein is then extracted from the diseased plant tissue.

<u>Three examples of risky viral experimentation</u>

Viral experimentation in general is a risky affair. Scientists in Australia accidentally created a potent strain of mousepox virus in an attempt to develop a sterilization technique for mice, a significant pest in Australia. This result was completely unexpected, because the virus was being used merely as a carrier to introduce the sterilization gene. Alarmed that the same technique could be applied to create a super-virulent strain of smallpox virus (a close relative of the

mousepox virus), the scientists issued a warning to the world's scientists, and also called for a strengthening of the Biological Weapons Convention (Nowak 2001; CSIRO Statement 2001). In a second example, a rabbit calicivirus being tested on an island off of Australia for use in controlling rabbit populations escaped the high-security containment facility, quickly spreading through much of Australia and New Zealand. No one is sure how it escaped, and while authorities claim there is no risk to human health, evidence of possible health impacts on humans is mounting (Hinds et al 1996, Smith et al 1998, pp. 18). Finally, Jesse Gelsinger, an 18-year old suffering from a rare liver disease, was treated with experimental gene therapy involving the use of a genetically engineered adenovirus as a vector to deliver the gene his body lacked. As with the mousepox case above, the virus – which was intended to serve merely as a means to introduce a foreign gene – killed its subject (Washington Post 1999).

These three examples demonstrate how genetic manipulation can change viruses in entirely unpredictable and potentially deadly ways, and the ease with which viruses in general can surmount any artificial barriers set up to contain them.

4.7.1 Plant viruses – still many unknowns

One concern is that genetically engineered plant virus vectors used to infect plants with biopharm genes could "cross over" and infect animals or humans. One study has found evidence that at some time in the past, a plant nanovirus crossed over to infect a vertebrate, possibly through exposure to sap from the infected plant. It then recombined with a vertebrate-infecting calicivirus (of the same family as the rabbit virus discussed in the second example above) (Gibbs & Weiller 1999). In answer to the self-posed question – "[c]ould plant viruses be involved in any clinical conditions, be they human or other animal?" – Dr. Charles Rupprecht, an expert on viruses with the Centers for Disease Control, gave this reply:

> "In fact, it wouldn't be too difficult to predict that now that we've got the tools available and given the realms of the majority of uncharacterized plant viruses that sooner or later somebody will put that connection together. It just hasn't been done yet." (Biologics Meeting I 2000, p. 126)

If this is true of plant viruses in general, an extra degree of caution is called for in open-air experiments involving their genetic manipulation, if they are to be permitted at all.

4.7.2 Open-air experiments with genetically engineered tobacco viruses

At least ten open-air experiments with viral-vectored plants to produce biopharmaceuticals have been conducted in the U.S. since 1991. In eight of the ten trials, the USDA kept the identity of the engineered drug genes secret as "confidential business information" of the company (see Section 6.3.1). In two trials conducted in 1991 and 1996, a highly toxic compound, trichosanthin, was produced in tobacco by means of a genetically engineered tobacco virus.

4.7.3 Case study of trichosanthin-producing tobacco

Trichosanthin is derived from the roots of a Chinese plant. It has a long history of use in China to induce abortions, and has been tested for use as an anti-AIDS and anti-cancer agent. Once considered promising for the treatment of AIDS, trichosanthin's toxicity – particularly severe

immune system responses upon repeated administrations – has limited its use for this purpose (Dharmananda, S). Effects associated with the intravenous use of trichosanthin include toxicity to embryos and fetuses (Chan et al 1993), renal toxicity (Ko & Tam 1994), neurological disorders (Kahn et al 1990), fever, headache, arthralgia and skin rashes (Dharmananda, S.). Health Canada, Canada's FDA, recently issued a warning against *ingestion* of two Chinese medications containing trichosanthin, ***"which is known to cause mutations in human cells and malformations in embryos, suppress the immune system, and produce severe allergic reactions."*** Health Canada noted that this substance is "highly toxic," and poses a "serious health hazard, particularly to children" (Health Canada 2001).

Trichosanthin was generated in tobacco plants by infecting them with a tobacco mosaic virus (TMV) engineered with the toxin's gene. TMV infects a wide range of plants, including tomato, pepper, eggplant, potato and numerous weeds (U of CT IPM 1998; NCSU PPE). It is also easily spread by touch, by plant debris carried on workers' clothing, or in tobacco products, which explains why tomato pickers are often prohibited from carrying or using tobacco. TMV can also be spread by contaminated farm implements or animals, and can survive the winter in many common weeds.

<u>The USDA ignores potential human health impacts of trichosanthin</u>

Despite the serious health risks of trichosanthin and the potential of its viral carrier (TMV) to infect food crops, the USDA's three-sentence "Impact on human health" section consists merely of bland assurances that the virus will not spread and the claim that there is "no evidence" of human health impacts from ingestion of trichosanthin, contrary to Health Canada's warning. No published literature is cited for the latter claim, only a "personal communication" with a single physician. The USDA does not even consider inhalant or dermal exposure, which would be the most likely routes in a field test involving the harvesting and processing of tobacco plants to extract the substance. And while the agency assumed without evidence that trichosanthin levels in the infected tobacco "should be below any significant level of biological activity," just 1 1/2 years later researchers reported ***"the highest accumulation of a foreign protein ever reported in any genetically engineered plant"*** for TMV-vectored trichosanthin in tobacco (Kumagai et al 1993, p. 429).

Other inadequacies include the brevity of the environmental assessment (only 17 pages of text), especially considering the fact that this was the first open-air release of any biopharm virus; the USDA's delegation of trial site monitoring responsibilities to the applicant, Biosource Technologies; and its general reliance on theoretical arguments and "personal communications" rather than hard data and published studies.

While only two field trials of trichosanthin-tobacco are reported by the USDA, other trials conducted by the same company with the same TMV-tobacco system in the same state as the 1996 trial (Kentucky) were carried out in 1998 and 1999 on 30 and 32 acres, respectively. Because the gene for these trials was kept secret as confidential business information, we do not know if trichosanthin was involved. See Appendix 4 for a fuller treatment of trichosanthin-producing tobacco.

4.8 Contraceptive Corn

A San Diego-based company called Epicyte is presently growing corn engineered to produce an antibody that binds to and disables sperm, with plans to market it as a topical contraceptive. Exploiting a rare condition known as immune infertility, Epicyte has taken the gene that codes for this anti-sperm antibody in infertile woman and spliced it into corn (McKie, R. 2001). According to information on Epicyte's website, the antibody is extremely potent, with only 10 mg required for a unit dose.

Epicyte does not report the expression level in its contraceptive corn. But based on data from other biopharm proteins, the company's projected demand for anti-sperm antibody of 5,000 kilograms a year would require cultivation of 6,500 to over 100,000 acres of contraceptive corn, assuming the antibody is recovered from corn kernels alone.[9]

What would be the human health, environmental, and economic implications of having tens of thousands of acres of corn growing anti-sperm antibody planted around the country? Some questions that present themselves include:

1) Will it be possible to prevent contamination of food-grade corn with such extensive cultivation?
2) Would consumption of contaminated corn impact human health? (An effective contraceptive dose of 10 mg of antibody could be contained in just 50 grams of raw corn – less than two ounces, based on avidin corn-level expression.)
3) What effects might this corn have on those most exposed to inhalation of pollen & grain dust as well as dermal contact: farmers, migrant farm-workers, corn-drug processors, etc.?
4) Would anti-sperm corn pose risks to wildlife, especially corn-eating mammals whose reproduction might be impacted?
5) What are the liability and export implications of contaminated corn?
6) How would crop debris – possibly containing drug residues – be disposed of? Would contraceptive corn be funneled into the food or feed chain after extraction? (see Section 4.3)
7) Who would regulate this mammoth enterprise, involving tens of thousands of acres of corn and possibly hundreds of farms? Will the FDA or USDA send out inspectors to ensure compliance with containment regulations? Will the company be left to police itself and its growers? What about theft of open-air corn and adulteration of the food supply?

4.9 Growth Factors and HIV/SIV proteins

Growth factors that have been experimentally grown in tobacco include erythropoietin and granulocyte macrophage colony stimulating factor (GM-CSF), which stimulate production of red and white blood cells, respectively (G. Giddings et al 2000, p. 1154). An engineered version of erythropoietin produced in mammalian cell culture (EPOGEN) is used to treat anemia resulting from chronic renal failure, treatment with the HIV drug zidovudine and chemotherapy (EPOGEN Label). GM-CSF is used to treat marrow stem-cell disorders and neutropenia, a

[9] Low-end expression estimate based on Cry9C in StarLink corn: 13 ppm of Cry9C in whole kernels of StarLink (SAP StarLink 2000b, p. 19); high-end expression estimate based on 200 ppm of avidin in kernels of avidin-producing corn (Hood et al 1997, p. 304). Other key assumptions: corn weighs 56 lbs. or 25.5 kg per bushel; one acre produces 150 bushels of corn. Calculations assume complete recovery.

condition involving abnormally low levels of white blood cells (G. Giddings et al, p. 1154; Quesenberry 1989).

gp120, a glycoprotein found on the surface of two strains of HIV and the closely related simian immunodeficiency virus (SIV), is being experimentally grown in corn and tobacco for possible development of an AIDS vaccine (ProdiGene HIV 2000; Miller 2000). Some scientists, however, believe that gp120 will never be effective as an AIDS vaccine, and might even suppress an effective immune response in vaccinated individuals (Koehler et al 2002). These scientists recommend basic research toward understanding the immune response to HIV-1 infection, and warn against the false hopes raised by premature vaccine trials, which can weaken the resolve to undertake infection prevention measures. Veljkovic et al (2001) call for a moratorium on clinical trials of HIV-1 gp120/160 vaccines.

4.9.1 Potential hazards of growth factors

Growth factors are extremely potent, able to exert powerful effects at just billionths of a gram. Several reports indicate that they can exhibit genetic toxicity (Lazutka, P. 1996), damage the DNA of human blood cells *in vitro* at concentrations as low as 50 billionths of a gram/ml (Liu et al 1998), induce changes characteristic of mutagens in the peripheral blood and/or bone marrow of mice (Yajima et al 1993a & 1993b & 1993c), and cross the placenta of pregnant mice to cause potentially dangerous alterations in the tissues of newborn pups (Kozlowski et al 1999). The maternal non-toxic dose of GM-CSF administered to pregnant cynomolgus monkeys caused abortions or embryonic deaths as well as prolonged genital bleeding, thrombus (blood clot) in the endometrium and necrosis in the chorionic villi of the mothers (Oneda et al 1998). The more serious side effects of EPOGEN treatment include hypertension and in some applications an increased incidence of blood clots. Erythropoietin and other growth factors also have the potential to promote the growth of tumors (EPOGEN Label).

Because these substances are normally administered intravenously, there is little experience with oral, dermal or inhalant exposure. But safety instructions for the handling of erythropoietin note that it "may be harmful by inhalation, ingestion or skin absorption. ... The toxicological properties have not been thoroughly investigated." Use of a respirator and chemical-resistant gloves is recommended (Erythropoietin MSDS). Similar recommendations apply to the handling of GM-CSF (GM-CSF MSDS). Due to the secrecy surrounding biopharm testing (see Section 6.3), it is unclear whether tobacco or other crops engineered with growth factors have been grown out-of-doors in field trials. An FDA representative also refused to say whether or not erythropoietin was being grown outdoors in plants (personal communication, Keith Webber), calling the information confidential, though this same FDA scientist has raised concerns about possible risks to the health of farmers posed by erythropoietin and other growth factors (Biologics Meeting II 2000, p. 95; see also Section 7.7). It would be unwise to permit open-air experimentation with crops growing such potent drugs because of their potential to cause serious harm to farm-workers and others through inhalation, ingestion or skin contact.

4.9.2 Hazards of gp120

Studies conducted to elucidate the mechanism of HIV infection have demonstrated that when injected into the rat brain, glycoprotein 120 [gp120] from the surface of the human

immunodeficiency virus causes fragmentation of DNA in brain cells (Bagetta et al 1995 & 1996), leading to apoptosis (i.e. "programmed cell death") of brain neurons (Corasaniti et al 2001). The dosages in these experiments were 100 nanograms [100 billionths of a gram] per rat per day over 7-14 days. Another study revealed similar DNA fragmentation and apoptosis of lymphocytes in the blood stream in response to gp120, an effect potentiated by the presence of cortisol, a stress hormone. This is thought to be one mechanism by which HIV overcomes the body's immune system and causes AIDS (Nair et al 2000).

gp120 from simian immunodeficiency virus has been grown in corn in the open air by ProdiGene in Nebraska (2001 season) and Hawaii (2002) under permit numbers 01-023-03r and 01-187-01r, respectively (see Appendix 6). There is no indication on the USDA website or in the USDA's response to a Friends of the Earth Freedom of Information Act request as to whether the government has conducted any environmental or health assessment.

4.10 Industrial enzymes

Another class of proteins that are being engineered into plants comprise enzymes intended for industrial uses in the production of food, detergents, paper, adhesives, pharmaceuticals and other products. These compounds are often required in large quantities, and in many cases are already produced at low cost by other means. Therefore, high expression levels are extremely important to make the plant production system competitive (ProdiGene Protein Products 1999). Some of these enzymes are known food, inhalant or dermal allergens that raise concerns especially for agricultural and other workers involved in their processing. One study carried out at a biotechnology plant producing industrial enzymes derived from recombinant bacteria and fungi discovered asthma and flu-like symptoms in 36 employees (Biagini et al 1996).

One of the enzymes experimentally produced in plants is alpha-amylase, which is used in the food industry to degrade starchy masses. Alpha-amylase from fungi has been found to cause allergies via inhalation at extremely low levels in the air – just 25 billionths of a gram per cubic meter (Baur et al 1998, p. 538). A closely related bacterial alpha-amylase was grown in transgenic alfalfa in field trials conducted in 1993 and 1994 by the University of Wisconsin. Another study that examined workers in the medical research, food, beverage, textile, tanning, cosmetic, pharmaceutical and detergent industries found that occupational asthma was caused by a wide variety of protein-degrading enzymes (Montanaro 1992).

4.10.1 Allergenic trypsin to be grown on hundreds of acres

Trypsin is a digestive enzyme produced by the pancreas of animals; it is traditionally obtained from cow or pig pancreas, and more recently from genetically engineered bacteria. Trypsin is used in biological research, in industrial applications, and to process certain pharmaceuticals (ProdiGene Trypsin 2002).

Trypsin-corn hybrids will reportedly be grown on hundreds of acres throughout the Midwest in 2002 by farmers under contract with ProdiGene (Des Moines Register 2002), making it the largest known planting of an industrial/pharmaceutical protein to date. ProdiGene plans to market hundreds of pounds of "non-animal-derived trypsin" by the end of 2002 and scale-up production to meet full market demand by 2003. It is estimated that the worldwide market for

trypsin will increase by five-fold in the next five years (ProdiGene Trypsin 2002), so hundreds of acres could quickly become thousands.

Open-air cultivation of trypsin-corn should not be permitted for several reasons:

1) Contamination of the food supply with trypsin is possible because: a) Corn pollen can travel for over a mile; b) Cultivation is planned in Cornbelt states; c) The large scale of cultivation increases the risk of contamination; d) As demonstrated by the StarLink corn debacle, contamination can occur by accidental mixing in the food supply, spilled seed, and many other mechanisms in addition to cross-pollination.

2) Trypsin is known to be highly allergenic, causing occupational asthma (a form of respiratory allergy) in pharmaceutical and industrial workers (Colten 1975, Colten & Strieder 1980, van Toorenenbergen et al 1991).

3) ProdiGene has reported extremely high concentrations of trypsin in its corn, with transient expression levels up to 19% of total soluble protein (ProdiGene Protease Patent 2000); these high levels increase the risk of asthma in farmers, farm-workers and processing workers.

4) Another corn contamination scandal a la StarLink could prove disastrous for farmers attempting to sell their corn overseas (see Section 7.5).

4.10.2 Other enzymes

Just a few of the many other plant-produced transgenic enzymes include cellulase, derived from a thermophilic bacteria and spliced into tobacco, which is intended for use in breaking down crop residues to produce alcohol (Dr. Jonathan Arias, U of Maryland, personal communication); manganese-dependent lignin peroxidase, derived from a fungus, for potential large-scale use in the paper industry (USDA Files 93-088-02 & 94-362-02); and beta-glucuronidase, which is used as a diagnostic reagent and a marker gene in the biotechnology industry (Stauffer Newsletter 2001b).

ProdiGene-Stauffer Seeds is testing a variety of corn that produces laccase, a lignin-degrading enzyme intended for use in the adhesives and textile industries (ProdiGene Laccase 2000). The laccase gene is derived from the mushroom *Trametes versicolor* (Permit No. 01-190-02)[10], a close relative of *Cryptococcus neoformans*, a fungus responsible for a life-threatening disease in AIDS patients known as cryptococcosis. In *C. neoformans,* laccase is thought to act as a virulence factor by oxidizing brain catecholamines such as dopamine (Williamson 1997). While laccase alone could not cause this disease, it might have other adverse effects. Laccase from the Japanese lacquer tree is obtainable from Sigma Chemical Company. A Material Safety Data Sheet on Sigma's website lists the following warnings for this laccase: "Acute Effects: May be harmful by inhalation, ingestion or skin absorption. Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals. The toxicological properties have not been thoroughly investigated." Those handling the substance are advised to wear a respirator,

[10] Search on the permit number in Appendix 6, or at the USDA website (www.nbiap.vt.edu/cfdocs/fieldtests1.cfm); the donor organism is listed as "turkey tails," the popular name for *Trametes versicolor*.

chemical-resistant gloves and other protective clothing (Laccase MSDS). Since humans do not possess the laccase enzyme (Williamson 1997, p. 99), its "foreignness" might help explain its allergenic potential. The *Trametes versicolor* laccase, from which ProdiGene's corn version is derived, also possesses potential N-linked glycosylation sites, another characteristic of many food allergens. ProdiGene/Stauffer believe that the potential market for their corn-produced laccase could require the planting of 200,000 to nearly 2 million acres of corn (Stauffer Letter 2001). Since the USDA provided no information on laccase-producing corn in its limited response to Friends of the Earth's FOIA request, we do not know if any health/environmental testing was conducted prior to its open-air release.

5. Environmental Impacts of Drug-Growing Plants

If nature could be made as neat and clean and predictable as a pharmaceutical factory, biopharming might not be such a bad idea. In this biotech fantasy world, the vagaries of nature would be abolished to achieve absolute quality control in the field. This is the fanciful image held by government regulators, as seen in a slide shown by the FDA's Michael Brennan at the Biologics Meeting in Ames two years ago, which symbolized the enterprise of biopharming with a "manufacturing plant in the field" (Biologics Meeting II 2000, p. 56). What would such an agriculture look like? Drug-growing plants engineered from scratch with artificial chromosomes, fields enclosed under miles-wide plastic domes, custom-manufactured soil or hydroponic solutions. Facilities surrounded by high-security fences, equipped with night lighting and alarms to protect against theft and adulteration. Access restricted to authorized personnel – "pharmers," company inspectors and government regulators. Our nation's biopharm fields would be transformed into high-security "industrial parks."

Most people would find such an agriculture abhorrent. But biopharm companies are not likely to spend the money to implement such containment and security measures, anyway. Given their huge R & D expenses, coupled with lack of marketable products, one of their top priorities is to minimize costs of production. The single most important imperative in minimizing costs is to secure regulatory approval to grow drug-plants ***in the open-air*** on a large-scale, commercial basis. Thus, for Carole Cramer of CropTech, a small Virginia company with plans to engineer 70,000 acres of tobacco (Richmond Times 2000, p. D15), the "real task" is to persuade regulators that plants engineered to manufacture drugs are no different than drug manufacturing plants:

> "So all of a sudden now, you're in a hugely uncontrolled environment. And the real task is to say that this environment is as safe and reproducible a source of pharmaceuticals as this [laboratory] environment." (Biologics Meeting I 2000, p. 48)

5.1 Drug Pollution

While Dr. Cramer's statement concerns the influence of environment on the quality of plant-grown pharmaceuticals, these substances may also have impacts on the environment. In a recent article entitled "Transgenic plants as factories for biopharmaceuticals," G. Giddings et al (2000, p. 1154) state that:

> "Biopharmaceuticals usually elicit responses at low concentrations, and may be toxic at higher ones. Many have physiochemical properties that might cause them to persist in the environment or bioaccumulate in living organisms, possibly damaging non-target organisms (they are environmentally persistent, lipophilic molecules that can pass through cellular membranes)."

Assessment of environmental impacts is hampered by several factors. First, the identity of most plant-grown biochemicals is kept secret as confidential business information (see Section 6.3.1). Secondly, even when the substance is known, there is often little or no information about the potential impacts of the more environmentally relevant routes of oral, dermal and inhalant exposure, because many plant-grown drugs are intended for intravenous use. Thirdly, both industry and government regulators (e.g. the USDA) rely extensively on claims of "no evidence of harm," suggesting positive evidence of no impacts, when in fact what is usually meant is that few or no pertinent studies have been undertaken (NAS 2002, p. 10).

The environmental impacts of a biopharmed substance will depend on many factors: the toxic, anti-nutritional or otherwise harmful properties of the pharmaceutical or biochemical, if any; the expression level in the host plant; the prevalence of the biopharm crop; and the compound's persistence in the environment.

5.1.1 Toxic, anti-nutritional and other harmful properties

We have reviewed some of the toxic properties of a few plant-grown compounds in Section 4 and Appendices 2-4. Groups of substances that may raise particular environmental concerns include: 1) Multiple use compounds with both pharmaceutical/research and insecticidal applications (e.g. avidin, aprotinin); 2) Substances intended for oral (edible vaccines) and/or dermal (anti-sperm antibody) use; 3) Extremely potent growth factors such as erythropoietin; 4) Toxins with broad-spectrum activity, such as the ribosomal inhibitor protein, trichosanthin.

5.1.2 Expression levels

As discussed in Section 4.4, the expression levels (i.e. concentrations) of plant-produced biopharmaceuticals are increasing, sometimes dramatically, as scientists learn the tricks of the genetic manipulation trade. For instance, insertion of transgenes in chloroplasts rather than the nucleus has yielded 20-fold higher levels of green fluorescent protein (Kleter et al 2000, 1.2.4) and 20 to 30-fold higher levels of the Bt protein Cry2Aa2 in tobacco (Kota et al 1999, pp. 1842-43). A substance with little or no impact at lower levels may cross the environmental impact threshold at higher levels.

5.1.3 Prevalence

The environmental impacts of drug and chemical crops will also depend on how widely they are planted. At this early stage, it is difficult to make even a rough estimate. But the industry has big plans. CropTech foresees a potential market of 70,000 acres for engineered tobacco (Richmond Times 2000). ProdiGene projects that 10% of the corn crop will be devoted to

biopharm production[11] by 2010 (L.A. Times 2001), and has already formulated detailed cost estimates for large-scale commercial production of various proteins (Evangelista et al 1998). While these hype-driven estimates of an industry badly in need of capital infusions must be taken with a large grain of salt (see Section 7.2.1), they cannot be totally discounted. Some crop-grown biopharmaceuticals such as human serum albumin are used in large quantities and could demand considerable acreage. As we saw in Section 4.8, tens of thousands of acres of corn would be required to meet the projected demand for a single product -- Epicyte's anti-sperm antibody.

5.1.4 Stability and persistence

Even though biopharmaceuticals are proteins, and are therefore generally expected to break down more rapidly than synthetic drugs, several plant-grown insecticidal and drug proteins have been shown to have surprising stability. For instance, the Bt protein produced by StarLink corn (Cry9C) is extremely stable to heat (2 hours at 100° C) and simulated gastric fluids (pH=2, pepsin) (Noteborn 1998). Other Cry proteins have been found to persist adsorbed to soil particles for hundreds of days (Saxena & Stotzky 2001b). The serine protease inhibitors discussed in Section 4.5.2 are also often hardy molecules. For instance: "Due to its structural configuration, aprotinin is relatively stable to high temperature, acids, alkali, organic solvents and proteolytic digestion" (Sigma Aprotinin). Trypsin is also relatively stable, proving resistant to all pancreatic serine proteases except elastase (Bernkop-Schnurch et al 2000).

Daughton & Ternes (1999) have shown that pharmaceuticals and personal care products are already polluting the environment, and may pose human health and environmental risks upon long-term exposure. They also point out that additive exposures to extremely low, sub-therapeutic doses of numerous drugs sharing a specific mode of action could lead to significant effects (p. 908). While their analysis was limited to non-biologic drugs, it may also apply to certain immunoactive compounds (e.g. vaccines), hormones (e.g. erythropoietin) and other potent biopharmaceuticals being grown in plants.

5.2 Impacts on Soil Ecosystems

Soil ecosystems are maintained through complex and subtle interactions between numerous species of bacteria, protozoa, fungi, nematodes, insects, earthworms and other creatures, in interplay with the soil matrix and root secretions (rhizosecretion) of plants. Very little is known about these interactions and how they contribute to healthy soil ecosystems. Without more baseline knowledge of soil ecology in the normal case with non-engineered plants, it will often be difficult to detect the unanticipated side effects of manipulating plant genomes, particularly in the rhizosphere (area surrounding the roots).

5.2.1 Rhizosecretion

Rhizosecretion is still a largely unstudied phenomenon, though it is estimated that plants secrete up to 10% of photosynthetically fixed carbon through their roots (Gleba et al 1999, p. 5974). The same researchers engineered tobacco to rhizosecrete three recombinant proteins into hydroponic

[11] Or "dual-use" production of crops for both drug extraction and food/feed byproduct – see Section 4.3.

media, and recommend this system as a promising alternative to extraction of biopharmaceuticals from field-grown plants (Ibid, p. 5976; see Appendix 5). A new variety of corn nearing commercialization is intentionally engineered for root secretions of a Bt toxin to kill a root pest, raising serious concerns about impacts on soil microbiota (Goldburg et al 2000, p. 13).

But even crops that are not specifically engineered for rhizosecretion can have "leaky" roots. Saxena & Stotzky (2000) discovered that transgenic Bt corn rhizosecretes the insecticidal toxin Cry1Ab, even though the developers did not intend this effect, and in fact it was not detected until years after the crop was commercialized.

Would root secretions of biopharmaceuticals or related compounds harm soil life? A key factor is whether the biopharm protein persists and accumulates in the soil. We do know that Cry1Ab, which is exuded from Bt corn roots and may also enter the soil through breakdown of crop debris, adheres to soil particles and persists in active form for at least 180 days (Stotzky, Progress 2000), indicating the potential for accumulation in the soil (Crecchio & Stotzky 2001).

In some cases, accumulation may not be necessary for harmful effects. In a study involving transgenic potatoes engineered with a T4 lysozyme gene to protect against a particular disease, the authors determined that the roots of potatoes expressing T4 lysozyme killed 1.5 to 3.5 times as many bacteria (*B. subtilis* as indicator species) as a control line. It is unclear whether these transgenic potatoes have harmful effects on other soil life beyond *B. subtilis* and the target pathogen (*Erwinia carotovora*) (Ahrenholtz et al 2000).

5.2.2 Crop residues

What happens to the engineered biochemical left in unused crop residues, such as stalks and roots? In the field trial situation, such material is burned or composted. If the crop residue is worked into the soil, the substance should be released upon degradation of the tissue matrix to join root exudates, possibly adding to the soil burden.

These fates could be altered due to unpredictable and unintended effects of the genetic engineering process. For instance, greater amounts of lignin have been found in several varieties of Bt corn (Saxena & Stotzky 2001a, p. 1705) and one line of soybeans manipulated for herbicide resistance (Coghlan 1999). Lignin – the woody substance in plants – is relatively indigestible. When added to soil, the high lignin Bt corn varieties were degraded more slowly than non-engineered varieties. Slower degradation could mean that the Bt toxin persists longer in the soil (Saxena & Stotzky 2001a, p. 1705). This is just one example of an unintended effect of genetic engineering, and its many possible consequences that happened to be detected. Whether biopharm crops would be affected in a similar manner or in other completely different ways that affect soil ecology cannot be predicted. However, the failure of industry and regulators to search for and detect such unintended effects in Generation One transgenic crops bodes ill for biopharm applications.

5.3 Impacts on Insects, Wildlife and Domesticated Animals

Biopharming in the open air will also impact life above ground and in the water. Insects and wildlife will pollinate, consume or otherwise interact with drug-growing plants, and aquatic life

will be exposed to any persistent residues washed into lakes and rivers. Because biopharmaceuticals are by definition designed to elicit responses of some sort in humans (e.g. immune or endocrine system), animals sharing common physiological features may respond similarly. Indeed, many human biopharmaceuticals are traditionally derived from mammals, such as insulin from cows and swine. Plant-grown animal drugs (e.g. transmissible gastroenteritis vaccine) may have a still higher probability of affecting related wildlife species than human proteins. A great many biopharmaceuticals are human enzymes (e.g. lysozymes), which often have closely related homologues, and in some cases activity, in animals, insects and even bacteria. Alternately, the plant-grown drug may have different effects on wildlife not related to its mode of action in humans (or domestic animals).

5.3.1 Insects

Several trends in biopharming raise serious concerns about harm to insects. First, several of the substances already commercialized or under development have multiple uses as drugs, research chemicals and/or insecticides. Second, several of these compounds are toxic to a broad range of insects due to their mechanisms of toxicity. Third, the drive to increase expression levels of mixed-use biopharmaceutical/insecticidal proteins will increase the risk to non-pest insect species.

Aprotinin, which is being grown experimentally in corn for use as a research chemical by ProdiGene (see Section 4.5.2 and Appendix 3), is a potent insecticide as well, causing 25% mortality in European corn borer larvae after 7 days of feeding with just 1.0 mg aprotinin per ml of feed. When combined with wheat germ agglutinin (WGA) at 0.2 mg/ml, this same amount of aprotinin causes 80% mortality in ECB larvae, exhibiting a powerful synergistic effect. Corn rootworm, the only other insect ProdiGene tested, experienced 60% mortality with 20 mg/ml aprotinin over 7 days (Aprotinin Patent 1998, Tables 3 & 4).

Aprotinin shortens the lives of honeybees

Aprotinin's toxic effects are not limited to pests. Several feeding experiments have shown that the lives of honeybees are significantly shortened at consumption levels as low as 18 μg aprotinin per day for seven days (Malone et al 2001, pp. 64-65; Burgess et al 1996; Malone et al 1995). Unfortunately, ProdiGene does not report the level of aprotinin, if any, in the pollen of its corn, although its use of the constitutive ubiquitin promoter makes pollen expression of aprotinin more likely (Zhong et al 1999, Figure 1, p. 346). We know, for instance, that the ubiquitin promoter in Herculex Cry1F corn drives Cry1F expression in Herculex corn pollen (EPA BRAD 2001b, pp. 6-7).

Avidin's "knockout punch" kills broad range of insects

Another multi-use insecticidal protein engineered into corn and expressed at a particularly high level is avidin, which is praised by USDA scientist Karl Kramer precisely for its broad-spectrum activity:

> "As a biopesticide, avidin is better than *Bacillus thuringiensis* (Bt) in corn because it has a knockout punch that hits a broader range of insects." (USDA Avidin 2000)

According to the NAS review committee, this "knockout punch" affects at least 26 different insect species (NAS 2002, p. 180). As noted in Section 4.5.1, avidin-corn could become widely used as a "background germplasm for production of other valuable recombinant proteins in corn" (USDA Avidin 2000).

Impacts on predators of insect herbivores should also be examined, especially given evidence of increased mortality in lacewings that consumed insect herbivore larvae fed either Bt plant material or purified Bt toxin (Hilbeck 1998a, 1998b & 1999).

5.3.2 Wildlife

Dr. Charles Rupprecht of the Centers of Disease Control raises a bewildering array of potential wildlife impacts that should be considered before further open-air cultivation of biopharm crops is permitted, even on a field-trial basis (Biologics Meeting I 2000, pp. 125-134). Substances that may pose particular concern are edible vaccines, both human and animal; contraceptive antibodies; potent growth factors (e.g. erythropoietin); anti-nutritional factors (e.g. avidin, protease inhibitors, lectins); proteases such as trypsin; and toxins such as trichosanthin. Dr. Rupprecht recommends detailed consideration of the following issues before the release of biopharm crops.

Containment conditions:
Should studies be conducted in a true island environment, an isolated ecosystem, in segregated plots or in unmarked fields? What about impacts on nearby land? Viral-vectored biopharm crops may need particularly strict controls due to the increased potential for contamination of other organisms (see Section 4.6).

Exposure over time and space
How long will non-target animals be exposed to the biopharm drug, and at what doses? Will the substance stay in place, or be moved by wind, water or other means? One might add, what is the potential for bioaccumulation up the food chain? These questions all become vastly more difficult to address as scale increases from field trials of several acres to commercial-scale plantings in the thousands of acres.

Testing of wildlife rather than just lab animals
Tests on highly-inbred lab animals (e.g. mice and rats) will not be adequate if one is truly interested in detecting potential effects on wildlife. Would model wildlife species be tested? If so, which ones? Rupprecht suggests field mice, raccoons and deer. Rupprecht's team tested over 40 vertebrates before the first release of recombinant rabies vaccine, while the USDA has required little or no animal testing of any sort in the few environmental assessments conducted to date for biopharm field trials.

What to look for and how to find it
Since much less is known about wildlife species than lab animals, the wildlife models would have to be thoroughly characterized to establish a "normal" baseline against which even subtle effects, such as weight loss, could be detected. Necropsies would be needed to detect gross, microscopic or ultra-structural lesions as well as physiological alterations, raising animal welfare

concerns. Age- and sex-specific effects would also need to be considered, particularly reproductive impacts on females (e.g. from anti-sperm antibody).

With vaccines and other immunoactive substances, particularly those designed for oral ingestion, one would have to look for antibody effects and immunosuppression (see Section 4.6.3). Could certain species of wildlife develop oral tolerance to an animal vaccine? Would they thus become susceptible to a pathogen to which they were previously resistant? These are urgent questions, especially since a corn-derived vaccine for transmissible gastroenteritis in swine is already in animal trials (ProdiGene TGEV 2000).

Dr. Rupprecht raises an important issue that, if taken seriously, casts doubt on the entire enterprise of open-air biopharming:

> "...what sort of call-back potentials there are if, in fact, we find out that maybe we did something wrong." (Ibid, p. 130)

5.3.3 Domesticated Animals

In large commercial plantings, there will be pressure to make "dual-use" of above-ground residues for animal feed (see Section 4.3). Cornstalks, for instance, are often used as forage material, and can form a major part of a farm animal's diet. Domesticated animals could thus have long-term exposure to substantial amounts of biopharmaceutical protein or insecticide, with potentially harmful effects depending on the substance. If the biopharm compound survives the ruminant digestive system intact, the biopharm protein could enter the soil through defecation, adding to the soil burden from breakdown of crop residues and "leaky" roots.

5.4 Inadvertent Biopharm Contamination

Thus far, we have only considered the potential environmental impacts of biopharm crops in the plots of earth where they are grown, as if they were unable to propagate and spread their unique traits to other living things. We have also ignored the contamination threat that arises from the rough and ready nature of the food system, which has developed to grow and move huge quantities of grains quickly and cheaply. There are many possible modes of biopharm contamination from seed purchase through field to table: seed spillage; residues of biopharm seeds in farm equipment; volunteer growth; cross-pollination by wind, insect or animal; and post-harvest mixing in the grain-handling system. (See Section 7.3.2 for a discussion of the various modes of seed dispersal.)

5.4.1 Biopharm contamination of food crops: corn

According to Dr. Norman Ellstrand, a geneticist at the University of California, Riverside who is a leading expert on pollen flow:

> "The field release of "third generation" transgenic crops that are grown to produce pharmaceutical and other industrial biochemicals will pose special challenges for containment if we do not want those chemicals appearing in the human food supply." (Ellstrand 2001)

These special containment challenges must be viewed in the context of two important developments: 1) Over the past several years, corn has emerged as by far the most popular crop for biopharmaceutical experimentation in the U.S., accounting for 134 of the 198 biopharm field trial notifications/permits listed by the USDA on its website; and 2) Two major contamination episodes highlight the propensity of transgenic corn to cross-pollinate with or otherwise contaminate conventional corn.

The unheeded lesson of StarLink

StarLink is a variety of transgenic corn engineered to produce Cry9C insecticidal toxin. Due to concerns that this toxin might cause allergies, the EPA approved StarLink in 1998 only for animal feed and industrial uses, not for human consumption. The EPA stipulated that StarLink could only be grown if: 1) A buffer strip 660 feet wide were planted around StarLink plots to mitigate pollen contamination of other corn; and 2) Both StarLink and buffer strip corn were segregated for distribution in non-food channels (EPA Cry9C Fact Sheet 2000). Despite these restrictions, StarLink contaminated a huge portion of the food supply. It was detected in 9-22% of grain samples tested by the USDA (Boston Globe 2001a & 2001b). The estimated number of people who consumed contaminated supermarket products (e.g. taco shells, bags of corn meal, etc.) is in the tens of millions (Freese 2001a, pp. 14-15). Hundreds of people who reported allergic reactions that they attributed to yellow-corn products were never tested (Ibid, p. 22). Numerous lawsuits to recover lost income due to this contamination scandal are still wending their way through the courts.

The extent of the contamination is startling when one considers that StarLink never represented more than 0.4% of U.S. corn acreage (EPA Preliminary Evaluation 2000, Table 5, p. 15). Most of the contamination was probably due to post-harvest mixing of StarLink with conventional corn. Another contributing factor is that some farmers were not informed of the planting and sales restrictions (Des Moines Register 2000). Yet evidence that popcorn, sweet corn, white corn and especially seed corn stocks were also contaminated with Cry9C strongly suggests that StarLink pollen blown by the wind fertilized conventional corn, despite the 660-foot border strip requirement (USDA News Release 2001, Hovey 2001).

The unheeded lesson of Oaxaca

In October and November of 2000, researchers in Oaxaca, Mexico detected transgenic DNA in five of seven samples of native corn. The findings were startling because the fields where these native "criollo" varieties grew were in a remote, mountainous area of the Sierra Norte de Oaxaca, 60 miles from the closest region where genetically engineered corn was ever known to have been planted. In addition, Mexico had imposed a moratorium on new plantings of transgenic corn in 1998. While one of the study's conclusions regarding reassortment of transgenic DNA has been contested, the fact that native Mexican corn has been contaminated by genetically engineered varieties is not in dispute.

The authors conclude that cross-pollination is more common than once thought, probably occurring at greater than expected distances, and that less remote areas will have higher levels of contamination. They also express concern that engineered corn could threaten the diversity of native varieties, especially in Mexico, the center of origin of the world's corn (Quist & Chapela 2001).

5.4.2 Biopharm pollution of food crops and weeds: canola

Canola is generally considered a poor choice for biopharming applications because it is insect-pollinated, outbreeds readily, and can potentially hybridize with a large number of wild relatives (Ellstrand et al 1999, pp. 549-50; RS Canada 2001, pp. 125-126; John Hammond of the USDA, Biologics Meeting I, pp. 116-117).

The unheeded lesson of triply-resistant canola

These gene flow concerns have been substantiated on the ground with the discovery in Canada of canola plants resistant to two and even three types of herbicide. Three types of canola, two genetically engineered and one mutated for resistance to a different herbicide each, are planted in Western Canada. According to The Royal Society of Canada (RS Canada 2001, p. 123), the gene flow events giving rise to the doubly- and triply-resistant plants are thought to have occurred through cross-pollination between different varieties, seeds accidentally transported via farm machinery, and/or seed spillage (e.g. blown from trucks transporting seeds to and from fields). In fact, "***it has been argued that seed spillage, a form of gene dispersal, may be a much more common mechanism resulting in hybridization between varieties than is likely by long-distance pollen flow by animal pollinators*** (McHughen 2000, p. 166)." (Ibid, p. 123)

This is no isolated problem. According to the Canadian experts:

> "Unfortunately, herbicide-resistant volunteer canola plants are beginning to develop into a major weed problem in some parts of the Prairie Provinces of Canada. Indeed, ***some weed scientists predict that volunteer canola could become one of Canada's most serious weed problems*** because of the large areas ... devoted to this crop." (Ibid, p. 122)

Biopharm canola could cause a similar problem by contaminating canola volunteers, which in turn could act as a long-term repository and genetic bridge for contamination of canola intended for human consumption by cross-pollination.

Blood thinner from leeches grown commercially in canola

Given this abundant evidence of outcrossing and explicit warnings by experts not to engineer canola for pharmaceutical production, it is surprising to note that one company is doing precisely that. SemBioSys of Calgary is presently growing canola engineered to produce hirudin, a blood thinner derived from leeches, on a commercial basis in Canada (G. Giddings et al 2000, p. 1154). No information about this food-drug hybrid was available on the website of SemBioSys. Hirudin is "a specific inhibitor of thrombin," which "inhibits coagulation in the initial stages and does not require the presence of other coagulation factors or plasma constituents" (Merck Index 1992). Interestingly, G. Giddings et al note that hirudin is presently produced in recombinant bacteria and yeast. Many other recombinant proteins can also be produced in such contained production systems (see Appendix 5).

One must ask whether the hirudin gene is being spread throughout the environment, whether food-grade canola and/or related weed species are presently producing hirudin blood thinner, and whether this drug is finding its way into the world's food supply. If it is not happening now, with presumably small areas under cultivation, what about future large scale plantings of this and

other biopharm canola plants? While biopharm experimentation with canola has thus far been limited to two field trials in the U.S., ProdiGene's patent on commercial production of protein-degrading enzymes in plants covers canola as well as corn, indicating interest in such applications for this crop in the U.S. (ProdiGene Protease Patent 2000).

5.4.3 Biopharm contamination of rice

To date, there have been nine known field trials of rice engineered to produce various pharmaceutical proteins in the U.S., including the blood thinner antithrombin III; antitrypsin, which belongs to the protease inhibitor class of proteins; serum albumin; and other pharm proteins kept secret as CBI.[12] Transgenic gene flow from rice can occur via cross-pollination, movement of seeds and possibly by horizontal gene transfer (from one species to another). While mostly a self-pollinator, rice is cited by The Royal Society of Canada as presenting a "moderate to high possibility" of outbreeding (RS Canada 2001, p. 125); some reports demonstrate up to 30 percent cross-pollination by wind (USDA EA 96-355-01). There are at least two species with which domesticated rice can interbreed in the U.S.: wild rice (*Oryza rufipogon*) and annual red rice (*Oryza sativa*). Wild rice is on the list of Federal Noxious Weeds (7 CFR 360) due to its ability to produce rhizomes and shatter (spread seeds) easily. "Annual red rice ... causes problems in rice fields because it is carried with cultivated rice and can significantly lower its value by reducing its processing characteristics" (USDA EA 96-355-01, pp. 5-6). According to geneticist Dr. Norman Ellstrand, genes from cultivated rice can easily be transferred by hybridization to red rice and other close relatives (Ellstrand et al 1999, p. 545). As with volunteer canola, the wild rice species could act as a repository and genetic bridge for the spread of biopharm genes to food-grade rice.

Despite the risk of outcrossing, biopharm field trial guidelines currently call for only a 20-foot isolation distance from other rice varieties (Biologics Meeting I 2000, p. 122). This isolation distance will be increased to 100 feet for biopharm rice trials in 2003 (USDA Guidance 2002). A sign of the laxness of USDA gene containment measures can be found in its approval for commercial cultivation of an herbicide-resistant variety of rice, AgrEvo's Liberty Link rice. Here, the USDA complacently admits that the gene conferring resistance to glufosinate herbicide will find its way to the weedy red rice:

> "It is assumed that the *bar* gene conferring tolerance to glufosinate will introgress into red rice and could result in a glufosinate-tolerant red rice population." (USDA FONSI 98-329-01; VII (E3). "However, these hybrid offspring will still be sensitive to other registered herbicides" (Ibid, Section V (C)).

The USDA notes that varieties of rice resistant to two other herbicides (imidazolinone and glyphosate) are under development (Ibid, Section VII (E3)). If introduced, exchange of herbicide-resistant traits between these three varieties and weedy red rice could lead to doubly- and triply-resistant red or volunteer rice, creating a weed problem analogous to the situation with canola discussed above. Given the apparent lack of concern for this problem, can we depend on the USDA to exercise any more caution with respect to pharmaceutical-producing rice?

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[12] Permit numbers 01-206-01, 01-029-02, 00-217-01, 00-069-01, 99-272-91, 99-272-92, 98-008-01, 97-363-01 and 96-355-01, all issued to Applied Phytologics.

5.4.4 Biopharm experimentation focused on crops most likely to outbreed

Given the abundant evidence of gene flow cited above for corn, canola and rice, one must ask why these crops are being "pharmed" at all. Overall, at least 149 of the 198 biopharm field trial permits/acknowledgements that have been issued by the USDA are for crops that pose a substantial risk of outcrossing. ***134 of the 198 involved wind-pollinated corn, which along with canola presents the highest risk of outcrossing*** (European Environment Agency 2002). Corn is the most popular biopharm plant because of its low cost, high protein content, and the existence of a well-developed storage, handling and distribution infrastructure. In other words, ***technical ease and especially economic considerations determine the choice of crop for biopharming, even if human health and the environment are thereby put at risk.***

The editors of Nature Biotechnology, the biotech industry's premier journal, recently underscored the threat of GMO contamination in unusually blunt terms:

> "Current gene-containment strategies cannot work reliably in the field. Seed companies will continue to confuse batches, and mills will continue to mix varieties. Although 'buffer zones' may theoretically control pollen dispersal (and gene spread), in practice farmers will be unable (or unwilling) to follow planting rules. Can we reasonably expect farmers to [clean] their agricultural equipment meticulously enough to remove all GM seed?
>
> Most seriously, gene flow (like mixing) could result in GM material unintended for human consumption ending up in the human food chain" (Nat Biotech 2002, p. 527).

5.5 Horizontal Gene Transfer

Another potential route for contamination of the environment with biopharmaceuticals is the transfer of genetic material from transgenic plants to bacteria or other unrelated organisms, one form of a phenomenon known as horizontal gene transfer (HGT). While no research has apparently been conducted on horizontal transfer of plant biopharm genes, several laboratory studies have demonstrated the transfer of antibiotic resistance genes, which are engineered into plant cells during the genetic manipulation process in order to permit selection of those cells that have incorporated the transgene.

5.5.1 Examples of horizontal gene transfer

Transgenic plant to soil bacteria

Horizontal transfer of genetic material is much more likely to occur if the recipient organism has DNA that contains sequences in common with (homologous to) the donor DNA. Transgenic plants are often engineered with segments of DNA derived from bacteria (e.g. antibiotic or herbicide resistance genes; promoter, termination and origin of replication sequences). This appears to increase the probability that associated DNA is transferred to bacteria (Gebhard & Smalla 1998). For instance, there have been successful transfers of antibiotic resistance genes to the soil bacteria *Acinetobacter* and *P. stutzeri* from extracts of transgenic plants, including potato, tobacco, sugar beet, canola and tomato (de Vries & Wackernagel 1998, 2002; Gebhard & Smalla 1998; de Vries et al 2001). While these transfers were accomplished under experimentally enhanced conditions, Michael Syvanen, an expert on horizontal gene transfer

(HGT), believes a similar frequency of HGT events could occur in a container storing tons of spoiling transgenic vegetables (Syvanen 2000).

Transgenic plant to soil fungus
Hoffmann et al (1994) reported transfer of an antibiotic resistance gene (hph gene for hygromycin resistance) from canola and several other transgenic plants to the fungus *Aspergillus niger* simply by growing the plant and fungus together.

Transgenic plant to intestinal organisms in honeybees
It is reported that German zoologist Dr. Hans-Hinrich Kaatz has observed transfer of the herbicide-resistance gene from genetically engineered canola to bacteria and yeast residing in the intestines of honeybees (Barnett, A 2000), though this finding has yet to be confirmed.

Recombinant plasmid to oral bacteria
Mercer et al (1999) demonstrated the transfer of an erythromycin resistance gene from a recombinant plasmid[13] to *Streptococcus gordonii*, a bacterium naturally found in human saliva. This transfer occurred despite the fairly rapid degradation of the transgenic DNA by enzymes present in the mouth, indicating that prolonged survival of naked DNA is not required for such transfers. "These findings indicate that DNA released from bacteria or food sources within the mouth has the potential to transform naturally competent oral bacteria" (p. 6).

5.5.2 The tip of the iceberg?

It should be emphasized that the results cited above were obtained with just a few of the 40 species of bacteria that are known to be "transformable" – that is, able to incorporate foreign DNA from the environment (Gebhard & Smalla 1998, p. 1550). Yet because most bacteria have not been identified, these 40 species probably represent a small proportion of the transformable bacteria that exist in nature:

> "Bacteria that are susceptible neither to culture nor identification represent a significant proportion of existing microflora. Therefore, without available knowledge of these bacteria, it is not possible to assess the possibility, probability or consequences of their acquisition of genes or gene fragments" (FAO-WHO 2000, p. 12).

5.5.3 Will transformed bacteria survive?

While it is often assumed that acquisition of foreign DNA make the recipient bacteria unfit and hence unable to survive in competition with natural bacteria, a panel of international experts disagrees:

> "Should horizontal gene transfer from a genetically modified plant to bacteria occur, the gene (e.g. an antibiotic resistance gene) may alter the fitness of the recipient cell. A reduction in fitness may not provide sufficient selective pressure to eliminate the gene or gene fragment from the gene pool. The presence of this DNA in the cell population could then serve as a genetic reserve for the evolution of the recipient species." (Ibid, p. 12)

[13] Vehicle used to introduce foreign DNA in genetic engineering.

With the ubiquitous use of antibiotics in animal agriculture, antibiotic resistance may in fact ***increase*** the fitness of transformed bacteria, promoting spread of the resistance gene to other bacteria, including possibly pathogens, which would then become resistant to antibiotic therapy. Concerns such as these led first the British Medical Association (BMA 1999), then other expert bodies (e.g. FAO-WHO 2000, pp. 12-13), to recommend an end to the use of antibiotic resistance genes in genetic engineering. It remains to be seen whether biotechnology companies will comply.

5.5.4 Linkage to resistance genes may promote spread of biopharm traits

A biopharm plant will harbor a drug or chemical gene linked to a gene for resistance to an antibiotic or herbicide. Both the biopharm gene and resistance gene could be horizontally transferred to microbes, for instance the bacteria involved in the breakdown of rotting transgenic plant tissue. Antibiotic/herbicide resistance might confer a survival advantage on the recipient bacteria vis-à-vis untransformed bacteria. This could lead to propagation of the co-engineered drug/chemical trait in bacterial populations exposed to the pertinent antibiotic/herbicide in agricultural settings, where use of both is common. Bacterial expression of the drug/chemical gene could then have grave environmental consequences, depending on the properties of the substance, its expression level, persistence in the environment, and other factors. While horizontal transfer and successful integration of such a large amount of DNA (two genes) may be highly unlikely, scientists remind us that "even very infrequent transformation events can be highly significant if the transforming DNA bestows a selective advantage on the recipient" (Mercer et al 1999, p. 10; see also RS Canada 2001, pp. 114-15).

5.5.5 Foreign DNA to mouse cells

Several studies suggest that foreign DNA can also be incorporated directly into the mammalian genome. Schubbert et al (1997) found that when mice were fed M13 bacteriophage[14], fragments of the viral M13 DNA survived digestion, penetrated the intestinal wall, and reached the nuclei of white blood cells, spleen and liver cells. The maximal size of detected viral DNA fragments was 976 bp (bloodstream), 1700 bp (feces) and 1299 bp (spleen). In another study, bacteriophage M13 and transgenic DNA (green fluorescent protein) fed to pregnant mice were found to cross the placental barrier and reach the cell nuclei of fetal and newborn mice (Schubbert et al 1998). Other studies have indicated that the integration of foreign DNA into mouse cells can change the very structure of chromatin far from the site of insertion, suggesting that the activity of many genes might be affected (Remus et al 1999, p. 1021).

The direct uptake and incorporation of foreign DNA by mammalian cells apparently occurs with use of "a highly flexible set of mechanisms" that do not require significant similarity between the recipient and donor DNA (Doerfler et al 2001, p. 279). This latter finding is particularly troubling, because it may open the door to mammalian incorporation of a wide range of foreign DNA, and raises the possibility that biopharmaceutical genes, too, could be integrated into the mammalian genome. Since the vast majority of ingested foreign DNA in food is broken down and never passes into the bloodstream, more research is needed to determine the causes and consequences of mammalian cells incorporating foreign DNA.

[14] A bacteriophage (abbreviated "phage") is a virus that infects bacteria.

5.5.6 Engineering the chloroplast increases risk of horizontal gene transfer

Genetic engineering of the chloroplast, which contains its own genetic material, rather than nuclear DNA has aroused a great deal of interest because it offers the possibility of vastly greater concentrations of foreign proteins (Heifetz 2000; see also Section 5.1.2). Each leaf cell of the tobacco plant, for instance, contains 5,000-10,000 chloroplasts. Since experiments show that foreign genes have been successfully integrated into all of the chloroplast genomes (Daniell et al 1998 & 2001), these chloroplast-transformed plants will carry roughly 5-10,000 times as much foreign DNA as standard nucleus-transformed plants, at least in leaf tissue. All other things being equal, when plant material containing this vastly increased level of foreign DNA is consumed or degrades in the environment, the frequency of horizontal gene transfer events involving the released DNA should increase proportionally, by three to four orders of magnitude. Chloroplast-integrated biopharm genes would thus have an increased potential to transfer to the genomes of gut bacteria, for example, or to integrate into mammalian cells as discussed in Section 5.5.5.

Chloroplast transformation has already been applied experimentally by Monsanto scientists to produce recombinant human growth hormone (rHGH) in tobacco chloroplasts (Staub et al 2000). The expression level of 7% of total soluble protein is reported to be more than 300-fold higher than that obtained with nuclear transformation.

Much more research is needed into the mechanisms of horizontal gene transfer before an informed assessment can be made of the potential for biopharmaceutical genes to be spread throughout the environment and indeed, into the genomes of mammals, humans, or the bacteria that inhabit them.

5.6 Experimental mechanisms to reduce contamination

Biotech companies are experimenting with several genetic mechanisms designed to reduce the likelihood that the transgene or its protein product will contaminate the environment. While often presented as "foolproof" containment mechanisms, it should be noted that these systems are experimental, in some cases arose accidentally, and have not been proven effective in commercial applications. Like other genetic manipulations, molecular gene containment measures will be subject to modification or failure under environmental stress.

5.6.1 Terminator

Terminator is the popular name for a complex set of experimental genetic manipulations which render seeds sterile through production of a toxin that kills the seed embryo. Developed by the USDA as a way to prevent "unauthorized regeneration" of seeds with patented engineered traits (USDA Terminator 2000), one proposed application is to prevent the spread of pharmaceutical and other co-engineered genes (Biologics Meeting I 2000, pp. 119, 122). However, if pollen containing the combination of Terminator and biopharm genes were to fertilize a corn plant destined for food use, the resulting kernels, while sterile, would still contain the pharmaceutical. Terminator would also not prevent horizontal transfer of the biopharm gene, or of its own elements. Its one advantage, ***assuming it functions correctly***, would be to reduce the risk of

volunteer biopharm plants from dispersed seed. However, Daniell (2002, p. 584) describes several ways in which Terminator could malfunction: 1) The three Terminator genes could become unlinked from each other, or from the gene of interest (e.g. biopharm), during reproduction; 2) The inducer applied to activate Terminator may not penetrate all the seeds; 3) A promoter might be "silenced" (i.e. deactivated), preventing expression of the sterilizing toxin. In all cases, fertile seeds could result.

Two genetic components of Terminator raise serious concerns in their own right: 1) A DNA-splicing enzyme (recombinase) that has already been shown to scramble mouse DNA and so render mouse sperm sterile (Schmidt et al 2000); and 2) Barnase, the Terminator toxin that indiscriminately breaks down RNA. Barnase is harmful to all cells, and has been shown to cause damage when perfused into rat kidneys (Ilinskaya & Vamvakas 1997).

Both the number and size of recombinase-plant field trials have increased dramatically in the last several years. Monsanto, which pledged not to commercialize Terminator (Monsanto Pledge 2000), has 17 field trials of recombinase-producing corn on a total of 286 acres in just 2001 and 2002 (USDA Field Trial Website). The average field trial size of 17 acres dwarfs past trials, which were all less than 1.2 acres, and indicates the company may be scaling up for commercialization. The use of corn, the favored biopharmaceutical host plant, suggests that the intended application may be Terminator-pharm gene combinations, though recombinase is also being tested for use in excising selectable marker genes from transgenic plants (Hare & Chua 2002).

According to USDA spokesman Willard Phelps, the chief application of Terminator is "to increase the value of proprietary seeds owned by US seed companies and to open up new markets in Second and Third World countries" (as quoted by RAFI 1998). In other words, Terminator will prevent farmers from saving seeds with patented traits, forcing them to buy new seed each year. As private seed companies selling Terminator seeds push public sector breeding efforts into the background, farmers around the world will have fewer and fewer non-sterile choices. For all of these reasons, this technology should not be further developed for any purpose.

5.6.2 Male sterility

Engineering a pharmaceutical plant to make its pollen sterile is another means to reduce the likelihood of genetic contamination. However, pollen-sterility systems are inevitably "leaky," meaning that not all pollen from such a plant is necessarily sterile (Ho et al 2001). One example is avidin corn, which ProdiGene (its developer) and the USDA claim to be male sterile (NAS 2002, p. 181). Yet close examination of the seminal paper on avidin corn reveals that only 82% of avidin-expressing plants are in fact male sterile, while 15% have "limited fertility" and 3% are fully fertile (Hood et al 1997, Table 2, p. 297). In addition, ProdiGene has not elucidated the mechanism of this partial sterility; it was an unintended effect of the genetic engineering process (see Appendix 2).

A Terminator-like system has been developed by Plant Genetic Systems in rapeseed to render its pollen sterile (Daniell 2002, p. 583). However, both Daniell and Ho et al (2001) note that pollen-sterile plants could still be fertilized by pollen from wild relatives or non-engineered

crops, and thus produce seeds that would sprout plants with viable pollen that could pass the GM (e.g. biopharm) trait in the next generation. These examples illustrate that male sterility is not a reliable genetic containment mechanism for biopharm plants.

5.6.3 Tissue-preferred promoters

Promoters are the "on-switches" used in genetic engineering to force plants to produce the foreign protein. Most promoters are "constitutive" – that is, they drive production of the transgenic protein in all tissues of the plant. Examples include the 35S cauliflower mosaic virus and corn ubiquitin promoters. Ubiquitin promoters are used in ProdiGene's avidin- and aprotinin-corn (Hood et al 1997; Zhong et al, 1999).

Developers of pharm crops would like to target production of their drug proteins to particular tissues through the use of so-called "tissue-specific" promoters to mitigate human health and environmental risks of the engineered drug. Despite many years of research in this area, however, tissue-specific expression has proven to be extremely difficult, so much so that ProdiGene uses the term "tissue-preferred," admitting that "some expression may occur in other parts of the plant" (Aprotinin Patent 1998; personal communication, John Howard of ProdiGene, 5/7/02).

There are a number of factors that can cause a "tissue-specific" promoter to drive expression of recombinant proteins in tissues other than the target organ. They include metabolite levels (Bevan et al 1993), the presence of exogenous sucrose (Jefferson et al 1990), light and plastid development (Chavez-Barcenas et al 2000), and methylation of the transgene (Cocciolone et al 2001).

These examples illustrate that limiting expression of a biopharm protein to a particular tissue is a daunting challenge, particularly given the broad range of environmental conditions and genetic backgrounds likely to be encountered by biopharm plants and genes.

6. Regulation of Drug-Growing Plants

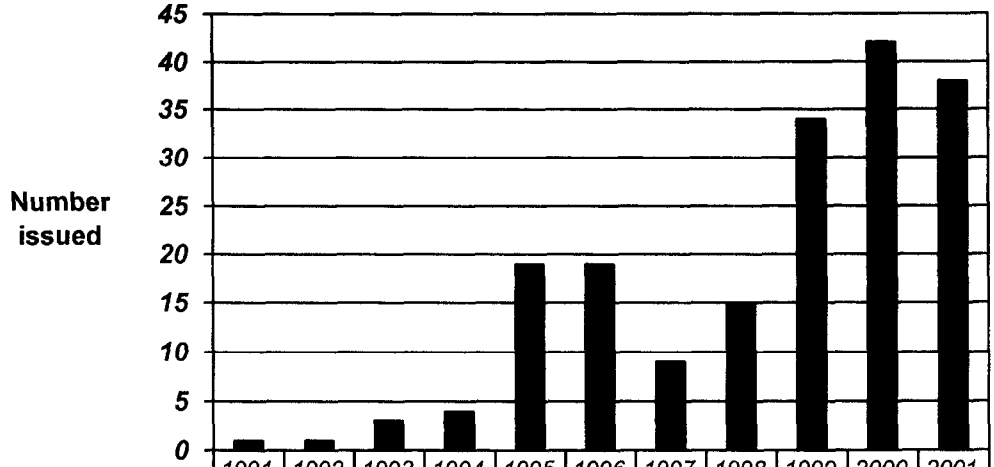
6.1 Overview of Application Process

The U.S. Department of Agriculture (USDA) treats biopharmaceutical-producing plants no differently than other genetically engineered crops. When a company wants to conduct a biopharm field trial, it submits a short application to the USDA's Animal and Plant Health Inspection Service (APHIS). The application contains basic information about the genetically engineered crop, its biopharmaceutical gene, the field trial site and design, starting and ending dates of the field trial, and the planned method for disposal of the crop. APHIS rarely conducts an environmental assessment for biopharm plants; the most recent dates to 1998 (USDA FOIA response 2001; personal communication, James White, USDA). APHIS then sends a short preliminary review of the application (not the application itself) to the department of agriculture of the state where the field trial is to be conducted. State officials must send comments to APHIS within 30 days. As of June 18, 2002, only 3 applications had been denied. The USDA gives no reason for the denials on its website.

While most biopharm plantings require a permit, some, such as avidin corn, are eligible for the streamlined "notification" procedure. In these cases, APHIS requires even less information and exercises less oversight. APHIS responds to notifications by issuing an "acknowledgement" within 30 days of receipt of the application.

6.2 Biopharm Field Trials: What We Know

A total of 198 permits/acknowledgments have been issued for field trials of biopharmaceuticals and biochemicals from the time open-air testing began in 1991 to June 18, 2002 (Appendix 6). These are the permits listed under the phenotypes of pharmaceutical protein, industrial enzyme(s), antibody and novel protein on the USDA's biotechnology website (www.nbiap.vt.edu/cfdocs/fieldtests1.cfm). The total number of field trials in this period is 315. There are more field trials than permits/acknowledgments because some of the latter cover trials in several states. As shown in Figure 1, interest has picked up over the past three years, with 64% of biopharm field permits/acknowledgments issued from 1999-2002.



	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
No. of permits	1	1	3	4	19	19	9	15	34	42	38

Figure 1: Biopharm permits/acknowledgments issued by the U.S. Department of Agriculture from 1991 through 2001. 12 additional permits/acknowl's issued from 1/1/02 to 6/18/02.

The 183 field trials for which acreage is reported range in size from less than 1 acre to 40 acres, with an average size of 4.9 acres. But since APHIS sets no limits on the size of field trials, some of the 132 trials for which acreage is ***not*** reported might be larger. The total reported acreage is 900 acres; total estimated acreage, based on the average size of trials for which area is reported, is 1550 acres. If commercial use of pharmaceutical and enzyme plants becomes more common, field trial sizes will increase. For instance, at least one trial planned for 2002 was recently reported in the press (not by APHIS) to be several hundred acres (see Section 4.10.1).

Tables 3 lists all biopharm field trials by state; Table 4 breaks down biopharm permits by crop.

State	No. of Field Trials
Nebraska	37
Hawaii	36
Puerto Rico	35
Wisconsin	27
Iowa	20
Florida	14
Illinois	14
Texas	13
California	11
Maryland	11
Kentucky	10
Indiana	9
Virginia	8
Minnesota	7
Missouri	6
Arkansas	5
Kansas	5
Michigan	4
North Carolina	4
Ohio	4
Washington	4
Delaware	3
Idaho	3
North Dakota	3
Oklahoma	3
Pennsylvania	3
South Dakota	3
Tennessee	3
Colorado	2
Georgia	2
Alabama	1
Arizona	1
Louisiana	1
New Jersey	1
Oregon	1
South Carolina	1
TOTAL	**315**

***Table 3** (above): Biopharm field trial states: 1991 to June 18, 2002;*

Crop	No. of permits/ acknowledgments
Corn	134
Soybeans	22
Viral-vectored tobacco	10
Rice	9
Tobacco	9
Alfalfa	4
Barley	3
Rapeseed (canola)	2
Wheat	2
Tomato	1
Safflower	1
Sugarcane	1
TOTAL	**198**

***Table 4** (above): Crops used for open-air biopharm experimentation, 1991 to January 31, 2002*

Institution	No. of permits/ acknowledgments
ProdiGene	85
Monsanto & Agracetus[1]	44
Pioneer	19
Applied Phytologics	13
Large Scale Biology & Biosource[2]	10
CropTech	7
Limagrain	4
U. of Wisconsin	3
Dow	2
Iowa State University	2
Cargill, Emlay & Assoc., Hawaii Agr. Research Center, Horan Bros. Agr. Enterprises, Meristem Therapeutics, Noble Foundation, RJ Reynolds, Univ. of Kentucky, Washington State Univ.	1 each

***Table 5** (above): Institutions involved in biopharm experiments: 1991 to June 18, 2002*

[1] Monsanto took over Agracetus in 1996

[2] Biosource Technologies, Inc took over Large Scale Biology and assumed its name in 1999

Biopharm experimentation has taken place in 36 states. Hawaii, Puerto Rico and Florida are popular because their mild climates allow year-round field trials. Iowa, Illinois and other Midwestern Corn Belt states are favored because corn is by far the most-used crop for biopharm experimentation, accounting for over two-thirds of total permits. ProdiGene of College Station, Texas, is the top institution, with 43% of the total permits, nearly all for corn hybrids (Table 5). Monsanto and Agracetus grow biopharm corn and soybean, while Applied Phytologics specializes in rice. CropTech engineers tobacco with drugs, and Large Scale Biology focuses mostly on viral-vectored tobacco.

6.3 Intellectual Property versus Public's Right to Know

What we aren't told about biopharming far exceeds what we know. The degree of secrecy surrounding this enterprise is extraordinary, and it is chiefly due to the companies' desire to hide their patented genetic novelties – which are considered "intellectual property" – from competitors and the general public.

6.3.1 Confidential Business Information (CBI)

USDA does not reveal the location of any field trial (beyond citing the state), in contrast to the practice in many other countries. Britain and Australia, for instance, keep publicly accessible registers that give the precise locations of field trials (Reuters 2001a; GeneWatch UK 2001). Without this information, a farmer has no means of finding out whether open-air biopharm experiments are being conducted in his/her vicinity, and so no way to defend against potential contamination. The general public is also kept ignorant.

Even if people knew *where* the field trials were, in most cases they would not know *what* was being grown there. This is because the identity and/or source of the biopharmaceutical or biochemical gene(s) is almost always claimed as "confidential business information" (CBI) of the applicant. In fact, ***CBI is cited 362 times for the 198 permits considered here***. In 206 cases, the identity of a biopharm gene is kept secret as CBI; there are 156 cases in which even the gene donor is claimed as CBI. The pertinent company decides whether the gene's identity is to be kept secret from the public. The USDA's stated policy is to disclose this information on its website only if the company does not claim it as CBI, or if the firm had previously chosen to publicize the gene's identity in the media (personal communication, James White, USDA).

This excessive secrecy was criticized by an expert committee of the National Academy of Sciences (NAS) that recently reviewed the USDA's performance at regulating transgenic plants (NAS 2002, p. 177). The committee found that the broad use of CBI not only impairs the public's right to know, but also hampers scientific peer review of APHIS decisions:

> "The committee finds that the extent of confidential business information (CBI) in registrant documents sent to APHIS hampers external review and transparency of the decision-making process. Indeed, the committee often found it difficult to gather the information needed to write this report due to inaccessible CBI." (NAS 2002, Exec. Summ., p. 11)

One explanation offered by the committee is that "the agency is not working to provide as much information as possible to the public" (NAS 2002, p. 177). Even the size of a field trial is often kept secret on the grounds that it provides a clue as to how close the company is to commercialization (personal communication, James White, USDA).

6.3.2 USDA's inadequate response to Friends of the Earth FOIA request

On April 11, 2001, Friends of the Earth submitted a Freedom of Information Act (FOIA) request to APHIS for full documentation concerning 131 permits involving field trials of biopharmaceutical and related proteins. As of this writing in June 2002, over one year later, APHIS has responded with the files for just two permits for which no confidential business information was claimed (USDA FOIA response 2001). A second reply consisted of just 7 environmental assessments (EAs) – the only ones that were conducted. These seven were already available on the USDA website. In its replies, APHIS blames a backlog of prior FOIAs for the excessive delay in fulfilling our request. For more on USDA's environmental assessments, see Section 6.5.2.

6.3.3 Secrecy and theft in the field

One incident of apparent theft of biopharm seeds has already been reported. According to Chris Webster of the drug company Pfizer, speaking at the Ames conference:

> "I'd just like to ask whether there's been any consideration given to the physical security of these recombinant lines?
>
> It occurs to me that you may have tens, hundreds, or even thousands of acres growing these plants, and ***what's to really prevent strange people coming in and taking them away and growing them somewhere else***, which would be an impact on the intellectual property of the company, actually has profound regulatory considerations [sic] as well.
>
> ***We've seen it on the vaccine side where modified live seeds have wandered off and have appeared in other products***. (Biologics Meeting II 2000, p. 77, emphasis added)

Unfortunately, we learn nothing further about this incident of apparent theft. Did these vaccine seeds get into the food supply? Were they stolen for illegal cultivation? Did they get mixed into some farmer's seed stock and unwittingly planted with conventional plants?[15] Few of the participants at the Ames conference took any interest in the "profound regulatory" implications of this incident or biopharm theft in general. Joe Jilka of ProdiGene, for instance, was much more concerned with how to protect his company's intellectual property – in this case, corn engineered to produce a vaccine for transmissible gastroenteritis (TGEV) in pigs:

> "...I think especially if you grow a few acres of [biopharm] corn in Iowa, the best way to secure it is to grow it just like any other corn. In other words, the anonymity of it just completely hides it.

[15] Chris Webster declined to comment further on this incident in a telephone conversation, and didn't respond to an e-mail request for clarification.

> You know, our TGEV corn grown [sic] was up here by Story City right by the interstate, and no one could have ever seen it. To secure it and build a fence around it is essentially to put a sign on it and say, 'This is where it's at. Come and take me.'"
>
> Otherwise there's absolutely no way to tell what it is..." (Ibid II, p. 77)

Both industry representatives and regulators generally agreed with this strategy of secrecy. Former USDA official David Espeseth, who moderated this part of the meeting, summed up the consensus:

> "We think being a tree in the forest is the best place to hide it." (Ibid II, p. 79)

6.4 Deficiencies in APHIS Regulations & Operations

6.4.1 Regulations do not cover all transgenic plants

APHIS regulates only those transgenic plants that it regards as "plant pests," either because the recipient plant is a weedy species, or the DNA spliced into the plant is derived from a plant pest as defined in federal regulations (7 CFR 340.2). As a result, ***some transgenic plants that do not contain "plant pest" material but do express bioactive compounds may "escape APHIS oversight" unless the companies involved voluntarily choose to inform APHIS with so-called "courtesy" notifications*** (NAS 2002, p. 107). The reason for this loophole is that government officials have insisted on regulating genetically engineered crops as if they were not fundamentally different than conventional crops. This dubious doctrine of "substantial equivalence" was formulated for the convenience of industry, ignoring the potential for unintended and unpredictable effects from artificially introducing foreign genetic constructs into plant cells.

6.4.2 Toxic & insecticidal compounds can escape regulation

Another loophole in biotech regulations is that transgenic plants are regulated according to their ***intended use*** rather than their ***actual properties***. This is particularly important in the case of plant-grown compounds that have multiple applications as research chemicals, pesticides and/or pharmaceuticals. For instance, since 1994 APHIS has permitted ProdiGene to grow avidin corn under its cursory "notification" procedure, which involves practically no assessment of impacts on the environment or human health, and only minimal containment measures. Yet avidin is a potent and broad-spectrum insecticide which also has anti-nutritional effects on mammals and humans. Thus, both the EPA (which has jurisdiction over pesticides) and the FDA (which regulates food safety) should have closely scrutinized avidin-corn before it was ever approved for open-air planting. Yet because ProdiGene ***intends to use*** corn-grown avidin only as a research chemical, it has escaped regulation by the EPA and FDA. Unfortunately, ProdiGene's intention does not protect humans, mammals or insects from avidin's toxic effects. This explains why the NAS review committee strongly criticized APHIS's decision to allow cultivation of avidin corn under the weak notification system (NAS 2002, pp. 180-82). A similar case is ProdiGene's aprotinin corn. Aprotinin is a blood-clotting agent that also kills insects and has adverse effects on the human and animal pancreas. It, too, has apparently escaped formal

regulation by the FDA and EPA because it is sold as a research chemical. How many other such compounds are being grown with virtually no oversight thanks to this loophole?

6.4.3 USDA not competent to evaluate health risks of biopharm field trials

APHIS regulates biopharm and biotech crops under the Federal Plant Pest Act and the Federal Plant Quarantine Act, laws which were formulated exclusively to prevent adverse environmental impacts of newly introduced plants, not deal with the risks to human health of plants engineered to produce potent drugs. This would perhaps explain why APHIS's "assessment" of the potential human health impacts of viral-vectored trichosanthin consisted of little more than a personal communication with one physician (see Section 4.7.3 and Appendix 4). While in some cases the USDA or the company may consult informally with the Food and Drug Administration concerning the potential health risks of biopharm crop trials, there do not appear to be any regulations in place to mandate such consultations. FDA representatives contacted by telephone have been unwilling to discuss any possible role their agency has had in advising companies or the USDA on field trials involving aprotinin or trypsin (personal communications, Kathryn Stein, formerly of FDA, & Keith Webber, FDA).

6.4.4 Commercialization of "plant products"

Although APHIS regulations prohibit the commercial sale of biopharm ***plants*** grown in field trials, APHIS does permit commercialization of the ***plant products*** of biopharm field trials. This dubious distinction has permitted ProdiGene to market corn-grown avidin and beta-glucuronidase through Sigma Chemical Company. At least one other corn-grown compound – the industrial enzyme laccase – is reportedly being marketed by Genencor International (Stauffer Newsletter 2001a), and ProdiGene has plans to market hundreds of pounds of corn-grown trypsin (see Section 4.10.1).

One must question the wisdom of allowing commercial imperatives to enter into a field trial situation that should be exclusively devoted to research, especially to the detection of any adverse impacts the experimental crop may have. To take a concrete example, a single acre of avidin corn yields at least 765 grams of avidin (Hood et al 1997, p. 304)[16], worth over $7 million at Sigma Chemical Company's selling price of $46.30 per 5 mg. If an applicant such as ProdiGene were to discover that its chemical-bearing crop had negative impacts on non-target insects or animals (e.g. corn-loving wildlife such as raccoons), could one rely on it to report such findings to APHIS, given the substantial financial loss a termination of the permit might entail? This situation is aggravated by APHIS's failure to exercise any meaningful oversight of biopharm field trials (see Section 6.5).

6.4.5 "Minimization" of gene flow not adequate in a zero-contamination world

APHIS recommendations for field trials do not even set zero contamination as a goal for companies to achieve in theory, much less practice. According to an APHIS guidance document for industry on notifications: "you must take steps to minimize the likelihood of pollination and successful fertilization of receptive plants outside the field trial" (USDA Performance 2001,

[16] Over 20 g avidin from 100 kg corn seed. Assuming yield of 150 bushels per acre, and with 1 bushel = 25.5 kg, 150 b/a x 25.5 kg/b / 100 kg x 20 g = 765 grams/acre.

point 5). "Minimizing the likelihood" of biopharm contamination of crops destined for food use is not sufficient. Yet this language is at least honest, in that it indicates the virtual impossibility of ensuring zero contamination. APHIS performance standards recommend, but do not require, that field trial corn be separated by an isolation distance of 660 feet from conventional corn, the standard used by growers of certified seed (Ibid, point 5). According to Douglas Russell of Monsanto's Integrated Protein Technologies, this 660-foot isolation distance permits a contamination level of 0.1% due to wind-blown pollen, which is considered an acceptable level of purity for seed stock (Biologics Meeting I 2000, p. 39). Even the more stringent 1320-foot standard recommended for use in biopharm field trials will not completely prevent contamination (NAS 2002, p. 124; see also Section 5.4.1).

One example of APHIS's lax standards in this regard comes from the files for two field trials of hybrid alfalfa genetically engineered to produce two industrial enzymes, alpha-amylase and lignin peroxidase, conducted by the University of Wisconsin (USDA Files 93-088-02 & 94-362-02).[17] First, APHIS allowed the trial to proceed even after it had been informed of a nearby plot of non-engineered alfalfa "within 200 yards of the test site," despite the fact that the isolation distance recommended for maintaining the purity of hybrid alfalfa seed stock is 1320 feet (USDA Isolation Distances), more than double the distance used in this field trial. Secondly, APHIS signed off on a field trial plan that permitted open flowers, increasing the risk of cross-pollination, despite the objections of the Wisconsin Department of Agriculture. While these trials were fairly small, the willingness of APHIS to disregard state officials and bend its rules to suit the applicant is not reassuring.

On May 21, 2002, the USDA issued a new guidance document on gene confinement measures for field trials of crops producing pharmaceuticals in 2003 (it does not apply to trials in 2002) (USDA Guidance 2002). As before, the stated goal is to mitigate rather than completely prevent spread of biopharm traits to related food crops and weeds. In fact, a USDA spokesperson told the author that replacement of the formerly used term "containment" with "confinement" is meant to indicate the impossibility of completely preventing genetic contamination. Beginning in 2003, the isolation distances recommended for biopharm corn are 0.25 mile (with buffer strips) or 0.5 mile (no buffer strips); in addition, biopharm corn is supposed to be planted at least one mile from sites with corn grown for seed stock. (See Section 7.3.2 for a discussion of potential biopharm contamination via seed dispersal.) It is unclear how committed the USDA is to these guidelines. Speaking of the isolation distances for corn "of 660' for industrial enzymes and 1320' for pharmaceutical products," ProdiGene-Stauffer CEO Anthony Laos recently told farmers: "We will be dealing with these distances until we can gain regulatory approval to lessen or abandon these requirements altogether" (Stauffer Letter 2001).

6.4.6 No requirement to test for unintended effects

APHIS requests only that companies report any gross morphological or agronomic differences between transgenic plants and their conventional counterparts that are not directly attributed to the biopharmaceutical trait – alterations in features such as leaf morphology, pollen viability, seed germination rates, disease resistance, etc. (NAS 2002, p. 115). There is no required list of

[17] These are the only files obtained in the Freedom of Information Act request by Friends of the Earth; they involve permit numbers 93-088-02 and 94-362-02. See Section 6.3.2.

tests to detect such unintended effects; APHIS merely asks that any differences the company happens to observe be reported. Subtle unintended effects not visible to the naked eye are likely to go undetected and unreported. For example, the increased lignin content of Bt corn was first reported by independent scientists only after 5 years of large-scale commercial cultivation, not by APHIS during the field trials of these crops (see Section 5.2.2).

6.4.7 Impacts on wildlife, insects virtually ignored

Based on the few environmental assessments (EAs) conducted thus far, it appears that APHIS pays little or no attention to the potential negative effects of biopharm proteins on wildlife or insects. For instance, in 1998 Limagrain obtained permits to conduct four field trials of corn expressing either: 1) human hemoglobin; 2) human procollagen; 3) human serum albumin; or 4) rabies virus protein. The four virtually identical EAs written by APHIS contain the same two-sentence section on non-target organism impacts: "There is *no reason to believe* that the novel gene products expressed by these plants would have a significant impact on non-target organisms, including vertebrate or invertebrate species. Neither of the novel gene products *is known to be* toxic to organisms" (USDA EA 98-117-01 to 04, 1998, emphasis added). The one reference cited for this statement relates not to the novel proteins, but rather only the herbicide-resistance marker gene product that was co-engineered into the plants. In other words, APHIS neither found nor required submission of data on the potential hazards of the unique human proteins that were the object of these field trials, relying instead on speculation. The NAS committee strongly criticized "no evidence" arguments such as this, which APHIS uses frequently and which are usually based on failure to conduct any pertinent studies whatsoever, or even search the literature, rather than negative results from targeted studies (NAS 2002, Exec. Summ., pp. 10-11).

6.5 APHIS Not Equipped to Regulate Biopharm Field Trials

We have seen a few of the serious deficiencies in APHIS's regulation and guidance vis-à-vis pharmaceutical plants. Below we will address some of the operational problems that make it difficult for APHIS to adequately protect human health, the environment and farmers' interests from the likely effects of pharmaceutical plants grown in the open air. These deficiencies will become increasingly important if field trials become larger and more pharmaceutical "plant products" are commercialized.

6.5.1 Understaffing makes adequate review of permit applications impossible

APHIS's Biotechnology, Biologics and Environmental Protection unit (BBEP) currently reviews approximately 1,000 applications for field testing and deregulation of transgenic plants each year (NAS 2002, p. 1), meaning that a corresponding number of decisions must be made. With only 10 permanent personnel available to do this work, as well as conduct field inspection visits, at best an average of only two person-days are available for each decision. Thus, it is not surprising that the NAS review committee found that this understaffing "may detrimentally affect the rigor of the determinations" (see NAS 2002, p. 182, which contains a fuller discussion of this problem).

6.5.2 APHIS environmental assessments of poor quality

Perhaps the staff shortage also helps to explain why APHIS has conducted only seven environmental assessments (EAs) for the 131 biopharm permits requested by Friends of the Earth in its FOIA request (USDA FOIA response 2001), none for any permits granted later than 1998 (confirmed in personal communication with Jim White of USDA). ***The failure to conduct any EAs prior to issuing permits over the past 3 years is especially troubling when one considers that over 60% of total biopharm field trials were conducted over this period (1999-2001).*** It also stands in direct contradiction to the USDA's claim in a document on its website, "Background on the Environmental Releases Database," which states: "The agency reviews permit applications and prepares an Environmental Assessment (EA) in which the potential environmental impact of the release is evaluated" (USDA Background 2001). Failings found in some or all of APHIS's EAs include:

1) Extremely brief treatment: Six of the 7 EAs are 11-13 pages (1 ½ spacing) in length. Many important topics are dealt with off-handedly, in one to two sentences. There are very few references to published studies, and frequent unsupported claims that there is "no evidence" of harm.
2) Boilerplate: Four of the EAs (USDA EA 98-117-01 to 04, 1998) contain nearly duplicate text, differing only in the description of the gene. In one EA (USDA EA 98-120-01, 1998), the starting date for the field test is cited in different passages as June 1996 (incorrect) and July 1998. At one point, APHIS refers to "this Monsanto application," when in fact the applicant was Biosource Technologies, Inc. This suggests that an EA conducted for a 1996 field trial by Monsanto was carelessly amended to serve as the EA for a different field trial 2 years later.
3) Lack of security measures: Failure to specify security measures, probably because none are required or undertaken. For instance, the four Limagrain EAs state only that: "Adequate precautions will be taken to provide for the physical security of the test plots" (USDA EA 98-117-01 to 04, 1998). As we saw in Section 6.3.3, APHIS appears completely comfortable with the "security" provided by growing the crop near a highway in a plot intentionally left unmarked so as not to attract attention.
4) Impact on current agricultural practices: APHIS assumes without argument that there will be no impact on farming practices. Yet since the agency is content with measures to "minimize" rather than completely prevent gene flow (e.g. cross-pollination), contamination of crops destined for food use is probable, especially with wind-pollinated corn. As seen with StarLink, such contamination could have extremely serious consequences for farmers in terms of increased costs, lost exports and lower prices.

6.5.3 Little or no on-the-ground oversight by APHIS personnel

The chronic shortage of personnel helps explain why many field trials sites are never inspected for compliance, meaning that oversight is left in the hands of the registrant company and the contract farmer. Even biopharm plant trials that are supposedly subject to stricter permit regulatory standards only prescribe a single visit at the beginning of the trial, with supervision during and afterwards (e.g. removal of volunteer biopharm plants) left to the company (e.g. USDA EA 98-117-01 to 04, 1998). In addition, APHIS field personnel "are not all trained to understand the implications of the evaluations they are making" (NAS 2002, p. 182) due to lack of training in biotechnology, so even the few inspections that are carried out may not be worth

much. The registrant company is expected to supply all crucial information, most of which remains unverified. For instance, the registrant is depended upon to "provide APHIS and State regulatory officials with information on the location of the nearest corn plants which are not part of the test" (USDA EA 98-117-01 to 04, 1998), a crucial factor in evaluation of the risk of cross-pollination that should be confirmed by government regulators in every case.

6.5.4 Company reporting requirements and quality of reports

Companies are required to submit reports at the end of the trial, or annual reports for multi-year trials. Given the paucity of agency field inspections discussed above, and the fact that the few APHIS inspections that do take place occur at the start of the trial, ***these company reports are often the only source of information APHIS has on the progress and results of the field trial.*** It thus becomes extremely important to examine the quality of company self-regulation and reporting.

No requirements for oversight of field trials by company

In the permits for which we have information, APHIS does not set any requirements with respect to on-the-ground oversight by company officials. This is important because most drug-producing plants are grown *not* by the registrant firm, but by farmers it has hired for the purpose (e.g. for ProdiGene, see Stauffer ICS Program 2002; for CropTech, see Miller 2000). As a result, APHIS regulations would actually permit a field trial to be carried out entirely by the contract farmer, with registrant company officials appearing only at the end to gather data for a report.

Quality of company reports

Unfortunately, the FOIA materials provided to us thus far by APHIS contain no company field trial reports. The files for the two permits discussed in Section 6.4.5 contain a scientific paper that might be intended as a report (USDA Files 93-088-02 & 94-362-02). This paper addresses the molecular characteristics of the engineered plant, the performance of the alfalfa, unintended stunting of some engineered plants, etc. Yet there is no indication that the registrant (University of Wisconsin) conducted any testing to detect "all deleterious effects on ... nontarget organisms, or the environment," as stipulated in the supplemental conditions of the permits.

A 1995 review of 85 registrant-submitted reports on field tests of transgenic crops found them to be seriously deficient (Mellon & Rissler 1995, p. 96). In particular, these reports provided little or no assessment of important environmental concerns such as weedy characteristics of the transgenic plant, gene flow through cross-pollination, potential creation of new viruses or impacts on non-target insects.

In a 1995 review of the seven transgenic crop approvals that had been granted by that time, Drs. Colin Purrington and Joy Bergelson found that much of the data submitted by applicants came from critically flawed experiments. They also reported a "remarkable reliance" on claims unsupported by hard evidence (Purrington & Bergelson 1995).

6.5.5 APHIS fails to engage scientific community or public

Given APHIS's overwhelming workload, one would think the agency would be anxious to engage the scientific community to assist in its regulatory decision-making. Yet APHIS makes little effort to seek external scientific review or public comment (NAS 2002, pp. 168-75). In particular, "there is essentially no opportunity for either general public comment or external scientific review on notification decisions prior to a decision being made" (Ibid, p. 170).

6.5.6 Failure to involve farmers

There is also little evidence to suggest that the USDA has made any efforts to engage farmers, one of the groups most affected by the advent of pharmaceutical plants, in its policy-making or individual permit decisions. For instance, no farmers or farmer group representatives spoke at the Plant-Derived Biologics Meeting held in Ames, Iowa in April 2000. This meeting forms the basis for an important guidance document regulating pharmaceutical plants due out in May of 2002, and so should have involved farmers.

7. What Will Biopharming Mean for Farmers?

> "The potential appears unlimited. Farm crops of the future will do more than feed a world population that's expected to double in the next 50 years. Through genetic engineering and the use of crops and animals as molecular bio-factories, it will also improve the health of millions of men, women and children around the globe. These bio-based solutions will not only provide miracle cures but will help prevent diseases and infections from occurring" (Olson 1999).

It seems to be a law of nature. As American farmers become fewer and fewer, driven off the land by farmer-killing policies and technologies, their moral mission becomes ever more exalted. First, it was feeding the world; now, it's healing the sick with "miracle cures." If farming becomes any more virtuous... there may soon not be any more farmers left.

Although there are dissenting voices, industry, academia and government are generally closing ranks to promote a technology of which most farmers know very little. Yet it is not company managers, professors or regulators who will grow drug-plant hybrids -- and farmers, for the most part, have yet to be heard from. What will biopharming mean for the steadily dwindling ranks of American farmers?

According to industry, biopharming offers the following advantages:
1) Farmers will share in the high profits of the pharmaceutical industry;
2) Farmers can grow drug-plant hybrids without changing present cultivation practices;
3) Large amounts of land will be planted to biopharm crops, and so many will benefit.

In order to address these questions, it is important first to understand why industry has embraced biopharming so enthusiastically.

7.1 Farmers or Fermentation Tanks?

Growing engineered plants in the field represents just one "production system" among many for obtaining biopharmaceuticals. Traditionally, drugs such as insulin have been and still are extracted from animal tissues. Genetically engineered bacteria and yeast, as well as plant and animal cell cultures are used to grow biopharmaceuticals in fermentation tanks. Some smaller proteins can even be synthesized from scratch from their amino acid building blocks.

In contrast to these proven techniques, which are laboratory or factory-based, drug-plant hybrids are intended mainly for open-air cultivation by hired farmers. By contracting out the actual production of pharmaceutical proteins to farmers, companies hope to limit their role to extraction, purification, marketing and intellectual property protection, and thereby realize huge cost savings. This system offers at least three significant economic advantages to biotech companies:

1) **Expensive fermentation tanks are replaced by farmers' capital**
   As Joe Jilka of ProdiGene, one of the leading companies in this field, puts it:

   > "We can take advantage of the low-cost production system that's out there. There are no capital requirements for new fermentors. You essentially use the farmers' capital system." (Biologics Meeting II 2000, p. 8)

2) **Production facilities and employees are replaced by contract farmers**
   This gives companies the cheapest means to scale-up or scale-down production. If a particular drug is not approved or fails in the marketplace, the biopharm company could simply terminate its contract with the farmer growing that drug rather than idle expensive production facilities. Likewise, a successful drug would simply require more acreage rather than new facilities (ProdiGene Benefits 1999).

3) **Storage facilities are replaced by patented seed**
   It is hoped that seed, particularly corn kernels, will provide a natural, low-cost "storage facility" that reduces expenses that in other production systems are incurred on storage (Ibid).

As promising as biopharming sounds, the question of whether it will help farmers depends on many factors, including the amount of land devoted to biopharm production; the share of profits that go to farmers; the feasibility of this production system versus other methods; continued investment and government subsidies; the need for time-consuming and expensive changes in cultivation practices; who bears liability for contamination incidents; availability of insurance; export implications; loss of independence; and potential health impacts on farmers and farm-workers.

7.2 Will Biopharming be an Economic Boon to Farmers?

7.2.1 How many farmers could biopharming employ?

Some industry representatives are making very big predictions. Anthony Laos, the chairman and CEO of ProdiGene and Stauffer, claims that 10% of American corn (6-8 million acres) will be

biopharm by 2010 (L.A. Times 2001; Seed and Crops Digest 1998; SeedQuest 1998). CropTech is telling tobacco farmers that up to 70,000 acres of tobacco will be converted to biopharmaceutical production (Richmond Times 2000). On the other hand, more sober heads are projecting much lower acreage. Andrew Hiatt of Epicyte, a San Diego-based company producing antibodies in corn, is predicting that Epicyte will need fewer than 1,000 acres of corn to fill the demand for one of its major prospective products: an antibody to protect against herpes. Even this "less than 1,000 acres" assumes a market of millions of people (Olson 1999). William White of Monsanto's Integrated Protein Technologies (IPT) says that even a drug needed in large quantities could be produced on a few thousand acres, a mere blip compared with the 77 million acres of corn grown in the U.S. (NYT 2000). Even these more sober projections assume that technical obstacles and regulatory hurdles will be overcome. It is safe to say that biopharming will employ extremely few farmers in the near and middle term, while the long-term prospects of the industry are too uncertain to make any credible projections.

7.2.2 Will biopharming bring increased income to farmers?

ProdiGene notes that the pharmaceutical industry is rated the most profitable in America – with gross profit margins averaging 70% (ProdiGene Market Strategy). Jim Thornton, vice-president of the biopharm company Demegen, speaks of production costs of just "pennies per gram" for plant-grown biopharmaceuticals (as quoted in Olson 1999).

One has to wonder what kind of profit, if any, a farmer could make growing crops that produce drugs selling for pennies per gram – surely a pittance compared to the industry's 70% gross profit margin. While others dispute this pennies-a-gram estimate, it does indicate the industry's strong resolve to keep production costs, including payments to contract farmers, to an absolute minimum.

ProdiGene and partner Stauffer Seeds have commercialized only two of their corn-produced compounds, avidin and beta-glucuronidase; both sell on the small research chemical market (personal communication, John Howard of ProdiGene, 5/7/02). While Stauffer Seeds widely advertises the commercial success of one of these compounds (avidin), the entire production is apparently obtained from a single farmer growing less than 5 acres of avidin corn (NAS 2002, p. 181). According to ProdiGene's John Howard, avidin corn is not being grown at all in 2002 because there is a surplus of avidin from past years' plantings (personal communication, 5/7/02). According to Stauffer's literature, it is offering farmers a premium of up to $1.00/bushel to grow drug-corn hybrids, a premium of about 40% over Chicago Board of Trade (Figure 2; Seed and Crops Digest 1998). Elsewhere, however, Stauffer-ProdiGene CEO Anthony Laos admits: "we cannot guarantee acres or premiums" (Stauffer Letter 2001). Whether or not any premium actually being offered pays for the hidden costs of biopharming will be addressed below.

Here, it is important to keep in mind that the corn-grown compounds marketed up to this point are research chemicals, which do not require extensive testing. Before any human drug could be sold, it would have to pass an exhaustive and expensive battery of tests required by the FDA: animal toxicity testing, three stages of human clinical trials, and post-market surveillance (Merck Manual 1992, pp. 2640-42). Add to this the special difficulties encountered with this brand-new production system, and it becomes clear that biopharm companies will have to recoup a huge

Figure 2. Stauffer Seed ad in Midwestern newspaper telling farmers "No Change in Current Farming Practices" to grow corn expressing industrial and pharmaceutical proteins. Note also the promised premium of $1.00/bushel. Stauffer-ProdiGene CEO Anthony Laos recently admitted: "[W]e cannot guarantee acres or premiums" (Stauffer Letter 2001).

load of sunken costs, and thus will need to retain the lion's share of profits for themselves, even for a very profitable biopharm product.

7.2.3 Biopharming must compete with other production methods

Biopharming is still at the experimental stage; it must compete with several other proven as well as newer methods for producing drugs. Drawbacks often cited for biopharming are expensive purification from whole plant tissue; plant glycosylation patterns that present the risk of allergy; and contamination risks arising from open-air production (the StarLink factor). Other systems, while perhaps in some respects more expensive, have their own advantages. For instance, drugs are easier and cheaper to purify with rhizosecretion (engineering plants to secrete drugs through their roots) and cell-based methods; animal cell cultures produce biopharmaceuticals with mammalian glycosylation patterns, which are much less likely to cause allergies; and most importantly, all other methods of production take place in a controlled setting, greatly reducing contamination and hence liability risks.

7.2.4 Biopharming dependent on investors & subsidies

At present, most biopharm companies are dependent on large infusions of cash from investors banking on future products. This support could easily be withdrawn, or re-directed to other production systems, if biopharming continues to fail to deliver marketable products. The very first company to conduct a biopharm field trial in 1991, Large Scale Biology (formerly Biosource Technologies), still does not have a single marketable product (NYT 2000). ProdiGene, which is heavily dependent on venture capital, suffered a near-fatal funding drought after the StarLink contamination scandal (Newsweek International 2002). The industry is also heavily dependent on government subsidies. For instance, CropTech has received over $10 million from the government in its short 10-year history (The Roanoke Times 2000a), as well as a $2 million Virginia state loan from the tobacco lawsuit settlement funds (The Roanoke Times 2000b) – again, without a marketable product to show for its efforts. The USDA (Kramer et al 2000) and publicly funded state universities (Hood et al 1997) conduct joint biopharm research with scientists from companies like ProdiGene, in effect subsidizing this technology. Like private investments, state and federal subsidies could easily be withdrawn or re-directed.

7.3 The Hidden Costs of Biopharming

An ad put out by Stauffer Seeds (see Figure 2) to entice farmers to grow its biopharmaceutical corn hybrids makes a rather surprising claim:

"No Change in Current Farming Practices"

If this claim is true, then Stauffer Seed and its partner ProdiGene are in gross violation of USDA field trial permit standards, and their biopharmaceutical corn hybrids are undoubtedly contaminating neighboring crops with their drug genes, and thus getting mixed into the food supply.

We can only hope that this claim is industry hype designed to attract skeptical farmers, for biopharmaceutical-producing plants will in fact require costly and time-consuming changes in current farming practices – if there is to be any chance at all of reducing its many risks.

7.3.1 The costs of containing pollen flow

Corn is wind-pollinated, and "it is possible for corn pollen to move on the wind for more than a mile" (Nafziger, E.). Insect-pollinated canola is also highly promiscuous. Preventing contamination of food-grade crops requires strict pollen containment practices. In a field trial situation involving less than one to a few acres, corn plants are sometimes "detasseled" by hand, or small bags are placed over the tassel, to prevent gene flow (USDA Performance 2001). This introduces more labor costs than growing normal corn.

Another practice known as temporal isolation involves planting one's corn later than surrounding corn so that it does not become fertile until the surrounding plants have stopped pollinating (pollen shed occurs over several weeks). One risk of this practice is lower yields from planting too late in the season. Another is that it often does not work. Iowa farmer Laura Krouse recently discovered her 1903 world champion line of open-pollinated corn contaminated by engineered traits despite practicing temporal isolation. She has suffered a 50-75% drop in sales due to this genetic pollution (personal communication; Cedar Rapids Gazette 2001).

The current isolation distance used by seed companies to prevent cross-pollination of their seed stock is 660 feet, yet StarLink corn contaminated the seed stocks of 71 of 288 seed companies surveyed by the USDA (USDA News Release 2001). Presumably, these companies followed this industry-standard practice, but to no avail. Double this isolation distance, or 1320 feet, is recommended for biopharm field trials (NAS 2002, p. 124), but even this increased distance will not stop contamination. Noting that the 660-foot isolation distance allows a contamination level of 0.1%, the expert NAS committee states:

> "There is no reason to assume that absolute isolation should be attained at twice that distance. It is likely there would be some very low level of contamination of any corn grown at or near the 1,320-foot isolation distance from the test plots" (NAS 2002, p. 125)

Norman Ellstrand, a geneticist who is an expert in this area, says that long-distance pollen flow is poorly understood: "It's just not clear that setting a double distance is going to solve everything." (as quoted in NYT 2000). Efforts to contain pollen flow cost money in terms of labor (e.g. detasseling or bags) and/or profit lost from the need for large isolation distances or planting late in the season. Liability risks will be addressed in Section 7.4.

7.3.2 The costs of seed dispersal and control of volunteers

Another means for pharmaceutical genes to spread is through inadvertent seed dispersal. The Royal Society of Canada notes that seed spillage (e.g. seed blown from trucks carrying seeds to and from fields) may be a more common mechanism resulting in contamination than pollen flow (RS Canada 2001, p. 123). A second mode of seed dispersal is the movement of biopharm seed from field to field by farm machinery. Since most farmers would plant biopharm along with

conventional crops using the same equipment for each, thorough and time-consuming cleaning of farm machinery will be required. According to the editors of Nature Biotechnology:

> "...in practice, farmers will be unable (or unwilling) to follow planting rules. Can we reasonably expect farmers to [clean] their agricultural equipment meticulously enough to remove all GM seed?" (Nat Biotech 2002, p. 527).

A third mode of contamination arises from incomplete harvesting of biopharm crops. According to Smyth et al (2002):

> "There is no harvesting system in place in the world that is capable of containing all the seeds produced on a plot of land. Many factors can combine to result in a large number of seeds (> 10^3/acre [i.e. 1,000/acre]) remaining in the fields" (p. 538).

Biopharm plants that sprout the following spring from these seeds left in the field – so-called "volunteers" – could easily become mixed with or cross-pollinate the following season's crop. This is particularly a concern with crops that produce many seeds, such as tobacco (up to one million seeds per plant) and canola. The Royal Society of Canada speaks of "the inherent difficulties in the containment of genetic material in the context of normal farming practices in which literally millions of small seeds are produced and harvested over large areas of the landscape" (RS Canada 2001, p. 123). Once again, "no change in current farming practices" almost assures biopharmaceutical contamination. Controlling volunteer growth would require careful field inspections and/or heavy use of herbicides. Farmers would have to take extraordinary care in every phase of seed handling, meaning increased labor costs and perhaps dedicated equipment to prevent farm machinery carry-over to conventional fields. Companies will probably require careful documentation of the disposition of seeds, entailing paperwork.

7.3.3 Strict controls on pesticide and herbicide use

As noted earlier, the FDA is presently considering whether, at the very least, to bar use of pesticides and herbicides for a certain period before harvest. Since many synthetic pesticides are long-lived compounds, farmers might have to forego the use of some altogether. Any prohibition of pesticide use would raise concerns about compliance in the event of serious insect attack or weed infestation, especially if a farmer were reliant on biopharm crops for a substantial part of his/her income.

7.3.4 Expensive soil characterization and amendment techniques

There are also indications that biopharming might require the use of costly techniques to characterize, map and adjust the composition of each plot of soil. This so-called "precision agriculture" involves the collection of voluminous information, grid by grid, about the land's fertility, soil types, productivity and history of pesticide use, which will often require hiring outside consultants. This mountain of data is then plotted on digital maps constructed with use of the satellite-linked global positioning system (Des Moines Register 1998; The Economist 2000). One wonders how much this equipment and these services will cost, and whether all but larger, wealthy farmers could afford them.

In view of the costs – both in time and money – of the many changes in farming practices necessitated by biopharming, it becomes doubtful whether farmers will really benefit from this innovation. The Economist's conclusion bears careful consideration:

> "Farmers' salvation does not lie at the bottom of a test tube. What they need most of all are sensible farm policies." (The Economist 2000)

7.4 Liability

Whatever the costs associated with altering farming practices to fit the biopharm paradigm, they will likely pale in comparison to liability risks. Even today, after nearly a decade of commercial biotech crop production, neither courts nor regulators have bothered to establish clear rules to deal with the many liability concerns associated with GMOs. Due to the unique health and environmental risks of many plant-grown pharmaceuticals, the advent of biopharming will only exacerbate these general GMO liability concerns.

7.4.1 Liability from inadvertent contamination of food crops

Whether by wind, insect or human error, contamination of the food supply is inevitable if drug-plant hybrids such as corn continue to be grown in the open air. Whether or not biopharmaceutical contamination will have legal and economic consequences depends on both the rules established by federal regulators *and the response of consumers*. Consumer rejection of contaminated foods could have serious repercussions even if regulators judge such contamination to be unobjectionable. Regulators would seem to have three choices:

1) Permit unlimited levels of biopharm contaminant in a food crop through issuance of a ***tolerance exemption***
2) Permit limited levels of biopharm contaminant through issuance of a ***tolerance***
3) Zero tolerance of biopharmaceutical contamination

The first option – **the tolerance exemption** – would clearly make life easier for biopharm companies and the government. For instance, imagine that Farmer A's corn becomes contaminated with anti-sperm antibody through cross-pollination with contraceptive corn grown by Farmer B under contract with Company C[18]. If the FDA had granted a tolerance exemption for the anti-sperm antibody, such contamination would be permissible under law, and not considered *adulteration*. And yet, this nice legal distinction would not prevent Farmer A from suffering huge financial losses if he is unable to sell his crop due to people's unwillingness to consume corn products laced with contraceptive. Nor would it prevent Farmer B and Company C from being sued by Farmer A for damages. It should be noted that, aside from StarLink's Cry9C, all substances that have been genetically engineered into commercially approved biotech plants to date have been granted tolerance exemptions for food use.

The second option, **issuance of a tolerance,** would require an accurate and reliable test to determine whether biopharm contamination exceeded the tolerance limit. Aventis CropScience petitioned the EPA for issuance of such a tolerance after its StarLink corn was found

[18] At present, Company C would be Epicyte, of San Diego, California.

contaminating millions of food products. The tolerance petition was rejected on the advice of the EPA's expert scientific advisors, who said that: 1) Even low levels of StarLink's Cry9C might cause allergies; and 2) The tests used to measure Cry9C in processed foods were not reliable due to degradation caused by processing (SAP StarLink 2001). Thus, establishment of maximum permissible levels for contamination of foods with various biopharmaceuticals will likewise be made difficult or impossible by the inability to accurately measure levels of these substances in processed food products. Even if possible, consumers may object to any level of biopharm contamination in their food.

The third option of **zero tolerance** of biopharmaceutical contamination is, practically speaking, an impossible standard to meet. This is especially true for corn, the favored biopharm host plant, because each kernel is the product of fertilization by a different grain of pollen (Quist & Chapela 2001), making extremely low levels of contamination possible. Zero tolerance is unlikely to be adopted because it would expose the biopharm companies to the greatest degree of liability and result in endless adulteration incidents that would discredit biopharming in the eyes of the public.

7.4.2 Liability risk from substandard drug quality

Environmental extremes, drought, insect attack, variations in soil fertility and many other factors can impair the quality of proteins a plant produces. Such environmentally-induced alterations in a biopharm protein could render it unsafe, or at least unusable. Field trials conducted over several years under a limited range of environmental conditions will often not predict how the biopharm plant and its drug behave under less frequently encountered, extreme conditions. Who will bear liability in the event that a biopharm protein is rendered unsafe or unusable by environmental conditions beyond the grower's control? Could a grower's biopharm crop be rejected without payment?

7.4.3 Liability risk from accidental consumption or theft

As discussed in Section 6.3.3, government regulators apparently find it perfectly acceptable for companies to have their biopharm crops grown with absolutely no identification or protection of field trial sites. This is completely unacceptable. It means that neighboring farmers are intentionally kept ignorant of drug-plant hybrids growing nearby and thus of the potential for contamination of their fields and crops. The public-at-large is likewise kept ignorant. It also raises the possibility of accidental consumption. Without clear identification and security measures, a biopharm plant could enter the food supply through being accidentally harvested together with nearby food-use crops, or a child could pick and consume a vaccine-fruit or vegetable, unaware that it differs from normal ones.

The StarLink affair demonstrates the potential health impacts and liability arising from failure to properly segregate an engineered crop. In March of 2002, a federal judge announced plans to approve a $9 million settlement in a class-action lawsuit brought against food companies, Aventis and Garst Seed Company on behalf of consumers who allege that the StarLink corn in foods they ate triggered allergies (The Wall Street Journal 2002). If even a *potential* genetically engineered allergen in the food supply can drive million dollar lawsuits, prospective biopharmers and others would do well to consider their potential liability in the event that biopharm crops contaminate foods with prescription drugs.

In many cases, however, it will be impossible to maintain the secrecy of a biopharm plot. This raises the possibility of theft and illicit misuse. For instance, some unscrupulous person could discover that a certain corn field is growing corn that contains an experimental AIDS vaccine (see Section 4.9.2), then secretly harvest it and grow a clandestine plot of his own, hoping to reap the higher prices being offered for drug-bearing plants. Even if not successful at selling his home-grown drug-corn, the plants would likely be grown without pollen containment practices, and thus would be likely to cross-pollinate with food-grade corn. ***Already, Pfizer's Chris Webster has reported a case in which "modified live [vaccine] seeds have wandered off and have appeared in other products"*** (Biologics Meeting II 2000, p. 77).

Regulators seem to believe that legal sanctions will provide a sufficient deterrent against theft and illicit misuse (Biologics Meeting II 2000, p. 78). Yet there is no explanation as to how theft of biopharm plants grown outside in unmarked, unprotected plots would be detected. The risk is obviously much greater than it would be in a pharmaceutical plant or other contained facility, with guards, locks and security alarms. The FDA's Michael Brennan, with his slide showing a pharmaceutical plant in the field (Ibid II, p. 56), is engaging in wishful thinking. Plants that manufacture drugs are much more difficult to control and monitor than drug manufacturing plants.

One could perhaps imagine fences, barbed wire, lights and security guards for a small stand of plants growing a very valuable pharm product, but such measures will obviously become infeasible – both economically and logistically – for large plantings. Once again, we must ask who will bear liability for risks associated with the theft, intentional misuse or accidental consumption of pharmaceutical-producing plants?

7.4.4 Who bears the liability?

The cultivation of pharmaceutical crops exposes not just biopharmers, but all farmers, to an unprecedented degree of liability: lawsuits brought by neighboring farmers or the biopharm company, and even government sanctions (the discussion in this section is based mainly on Moeller 2001).

Farmer suing farmer

If a farmer's drug-crop contaminates a neighbor's field, the neighbor could sue on the basis of trespass, nuisance, negligence or strict liability. A person is strictly liable for engaging in abnormally dangerous activity, even if s/he is not reckless or negligent, and could be sued by anyone who suffers damage as a result. Some legal scholars hold that both farmer and GMO-seed company could be held strictly liable for damages in case their GMO-crop (e.g. biopharm) contaminates a neighbor's field. Such damages could include financial losses from inability to sell a contaminated crop or loss of organic status. In one case decided by the Washington State Supreme Court, an organic farmer successfully sued an aerial pesticide spray company for economic losses related to drift of sprayed pesticide onto his farm.

Contract liability

Biopharmers and their neighbors could be liable in a number of ways relating to breach of contract or infringement of a biopharm company's intellectual property rights. Many GMO-seed companies currently require farmers to sign "technology use agreements" that prohibit seed-saving as a means to protect their patented "intellectual property." Biopharming contracts will be similar, but will also contain detailed growing and seed-handling specifications intended to ensure the quality of the drug, prevent contamination of non-biopharm fields, etc. If a case can be made that such specifications were not strictly followed, the biopharmer could be liable for any damages that result.

Biopharming exposes all farmers to contract liability, whether they choose to grow these crops or not. A farmer under contract to supply organic, non-GMO, or even merely food-grade crops could find himself in breach of contract were his crops to be contaminated with biopharm traits. Such contamination could even occur through the farmer's unwitting purchase of seeds contaminated with biopharm traits. This latter possibility is illustrated by the extensive adulteration of seed stock with StarLink's Cry9C gene (USDA News Release 2001).

The lesson of Monsanto

Both biopharmers and other farmers could be sued by a biopharm company for the illicit presence of the company's patented gene in their crops. Anyone who doubts this should take a hard look at Monsanto, which has sued numerous farmers in North Dakota, South Dakota, Indiana, Louisiana and other states, as well as Canada, for supposedly saving and planting the company's patented Roundup Ready seeds illegally. According to farmers, the presence of the biotech trait was due to seed spillage from a passing truck, hard-to-control volunteer growth from a prior season's planting of GMO seed, or some other undetermined mechanism. An organic farmer in Canada, Percy Schmeiser, was sued by Monsanto for the presence of the company's Roundup Ready trait in his canola fields. Incredibly, the court ruled that it did not matter how Schmeiser's field became tainted, and compelled him to pay Monsanto $100,000 in damages. Schmeiser, who never grew Roundup Ready canola, thinks the contamination probably occurred through spillage of seed from a truck passing by his fields (see www.percyschmeiser.com).

Regulatory liability

Biopharmers and other farmers could also be exposed to enforcement actions brought against them by federal regulatory agencies for failure to comply with government-mandated restrictions. This is a murky area that will hopefully be clarified by a guidance document on biopharming that the FDA and USDA are supposed to issue in May of 2002. We do know that FDA and USDA regulators plan to reserve the right to inspect biopharm fields (Biologics Meeting II 2000, p. 56), and have authority to impose fines for theft of biopharm crops (Ibid II, p. 78).

Insurance companies leery

One reason biopharm companies will be anxious to shift liability to farmers is the fact that many insurance companies are leery of covering the unknown, unpredictable risks of genetic engineering. For instance, the Swiss re-insurance company Rueck concluded in 1998 that there was no satisfactory way to evaluate the risks of genetically engineered crops and thus offer

appropriate coverage (EMS 2000). In 1999, Maurice Pullen, an underwriting manager for Cigna International, warned business insurers of the risks of covering GMOs:

> "Our experience with asbestos, PCBs and other 'miracle' products in the past should have warned us of the potential dangers of diving into issues before we have an adequate awareness of the exposures" (as quoted in Guebert 1999)

Lesson of StarLink

These insurance company warnings all predate the StarLink contamination debacle, which has given rise to at least nine class action lawsuits in six states against Aventis CropScience, its developer (Moeller 2001, p. 2). While Aventis has tried to maintain that StarLink contamination was "unavoidable and unforeseeable" (Aventis Petition 2001, p. 62), the facts show otherwise. ***In one particularly unethical practice, Aventis and/or its chief seed dealer, Garst Seed Company, lied to farmers by printing fraudulent information on StarLink seed tags. These tags explicitly informed farmers that: "You are licensed upon purchase of this product only to produce forage or grain for food, feed or grain processing" (Des Moines Register 2000a, emphasis added), when in fact StarLink was not approved for human consumption.*** This blatant dishonesty, coupled with the publicity surrounding StarLink, explain why Aventis and Garst are being forced to pay compensation to farmers. Even so, some lawsuits are still pending, and it remains to be seen how well farmers will be compensated for their losses.

Liability risks to all farmers argue against open-air cultivation of pharm plants

As we have seen, the liability risks of biopharming affect not just those few who choose to grow these crops, but all farmers. Even with the strictest gene containment procedures, contamination of food-grade crops with drugs and industrial chemicals is likely, especially with wind-pollinated corn.

7.5 Will Biopharming Hurt American Exports?

Citizens in Europe, Asia and much of the rest of the world do not want to eat genetically engineered foods. That's why they have successfully pressured their governments to require listing of genetically engineered ingredients on food labels – so they can choose GMO-free products. As a result, American farmers have often found it difficult to sell their engineered crops abroad. If citizens overseas are already suspicious of existing GMOs, how will they react to news that pharmaceuticals have contaminated the American food supply?

7.5.1 The lesson of StarLink

The StarLink scandal provides a valuable lesson on how contamination of the food supply with a potentially dangerous genetically engineered substance can hurt farmers through reduced exports. According to Iowa State University Professor of Economics Dr. Robert Wisner, the fallout from StarLink contamination was chiefly responsible for the 7% decline in U.S. corn exports as of November 1, 2001 versus the preceding year. Sales to Japan, Korea and Taiwan, which together usually buy more than half of U.S. corn exports, fell by a full 20% (Des Moines Register 2001). These export losses are at least partially responsible for historically low corn prices.

Both the Japanese and the Koreans turned to Argentina, China and South Africa to make up for rejected American corn (Pro Farmer 2000; Reuters 2001c). Government and industry, however, were unconcerned about StarLink contamination, complacently assuming that foreign consumers had no choice but to buy U.S. corn (Des Moines Register 2000b).

StarLink offers at least two lessons for American farmers: 1) If even a *potential* genetically engineered allergen in the food supply caused such havoc, imagine how export markets would react to corn laced with a prescription drug; 2) The USDA, with its arrogant "they'll-buy-our-crops-no-matter-what" attitude, cannot be trusted to regulate biopharming safely.

7.5.2 Critical attitude to biotech foods overseas argues against biopharming

The more critical attitude to biotech crops overseas suggests that foreign consumers would strongly reject American crops contaminated with biopharmaceuticals. Even without biopharm contamination, it was recently reported that European consumers might not accept genetically engineered crops for 10 years (Safford, D. 2002). Even this projection may be optimistic, as many supermarkets in Britain have transitioned to non-GMO organic foods, and are even phasing out meat derived from animals fed genetically engineered grain – all in response to their customers' demands.

The U.S. government likes to portray foreign opposition to biotech foods as based on irrational fear of the unknown, or as a disguised form of protectionism. But in fact, popular opposition to GMOs is finding ever more support in the scientific community. Even pro-biotech scientific bodies are calling for better studies, more rigorous testing, less collusion between industry and government, and much stricter regulation of genetically engineered foods. Examples include the U.K. Royal Society (UK Royal Society 2002), the RIKILT Institute in the Netherlands (Kuiper et al 2001), and the Royal Society of Canada (RS Canada 2001).

7.5.3 What will biopharming mean for U.S. food exports?

Foreign grain traders, governments and citizens will not soon forget StarLink, nor that it was a variety of genetically engineered *corn*. It is not hard to believe that another corn contamination scandal – involving trypsin corn, perhaps, which is to be grown on hundreds of acres across the Midwest in 2002 (Des Moines Register 2002) – would forever turn many foreign customers, already burned by StarLink, away from U.S. corn altogether.

7.6 Loss of Independence

Biopharming represents the latest step on the road to an agricultural world where American farmers are hired hands rather than independent producers. Growers who rent out their "low-cost production systems" (Joe Jilka of ProdiGene) and labor to grow company-owned "intellectual property" (a.k.a. seeds) will probably be required to sign detailed contracts that specify exactly how a farmer is to farm (see Biologics Meeting I 2000, p. 72; II, p. 95). The farmer's knowledge, skill and judgement will be largely replaced by the biopharm company's "specifications," which will prescribe things like type and amount of fertilizer to use and when to apply it; plant spacing patterns; painstaking removal of all seeds from farm machinery and bins

to prevent contamination of conventional crops; strict pollen containment practices; type and amount of herbicides or pesticides to use and when (not) to apply them (if permitted at all); harvesting times and methods; storage conditions, etc. The farmer will also be expected to give precise accounting of every biopharm seed s/he plants, with possible liability implications if s/he is unable to do so.

7.7 Will Plant-Grown Drugs Endanger Farmers' Health?

Nearly 300 field trials of biopharmaceuticals and biochemicals have already been conducted in the U.S. In the majority of cases, the identity of the drug has been kept hidden from the public as "confidential business information." One farmer, speaking anonymously, said that a biopharm company told him it does not matter what is being grown, you'll make a dollar more per bushel. Field trial sites have also been kept secret.

Farmers, farm workers and biopharm company employees are more likely to suffer health impairment than the general public because their exposure is greater. Farmers will breath the pollen and dust of these crops and absorb the biopharmaceutical through their skin while handling and harvesting them; biopharm company employees involved in processing these plants will also have high exposure. What safety measures are planned to protect these front-line biopharm workers? Surprisingly, the entire 240-page transcript of the two-day "Plant-Derived Biologics Meeting" in Ames, Iowa contains only a single brief discussion on possible health risks to farmers:

> "I have a question, I suppose for the industry, that in talking about contracting out the growth of, for example, corn that produces a biologic, in some instances the products, if it's a growth factor like EPO [erythropoietin] – EPO or something like that, there might be safety issues to the farmer himself who inhaled the dust perhaps during the processing.
>
> "Do you foresee that when you contract out with the farmer that they would be apprised of what the product is that would be in the corn, or would that be a problem?" (Keith Webber, FDA, Biologics Meeting II 2000, p. 95)

Mr. Webber is to be commended for at least raising this issue, alone among the many government officials in attendance, but his remark suggests that ***it is the company's choice whether to even tell a farmer the identity of a potentially dangerous drug he is growing.***

This example of government's careless attitude towards farmer and farm-worker health is not an anomaly. Before and during the StarLink contamination scandal, the EPA's scientific advisors called repeatedly for someone to test Garst Seed Company workers for possible allergic reactions to StarLink corn, but ***neither the government nor Aventis did so*** (SAP StarLink 2000a, pp. 8-9; SAP StarLink 2000b, p. 26). Likewise, the government recently re-registered Bt crops without evaluating them for allergenic potential (Freese 2001b), despite the finding by an EPA-sponsored scientist that farm-workers exposed to closely related Bt sprays had antibody responses consistent with allergic reactions (Bernstein et al 1999).

7.8 Conclusion

The bottom line is that biopharming appears to offer very little to farmers in exchange for the risks it brings. The one advantage – small premiums over conventional crop prices – is more than offset by liability risks, expensive changes in farming practices, health impacts from handling and breathing drug-bearing plants, and the other factors discussed above.

Beyond these specific concerns, however, is the reputation of American farmers in export markets. Foreign grain traders in Europe and Japan have already warned the U.S. that they will not buy genetically engineered wheat, presently being developed by Monsanto (Reuters 2001b, Grand Forks Herald 2002). StarLink contamination has convinced many grain handlers and consumers in Asia and Europe that American corn is be avoided whenever possible. Drug contamination would harden that impression, and likely transform what are hopefully now only temporary losses in export sales into permanent ones. ***Thus, biopharming is likely to harm all farmers, whether they choose to grow drug-plant hybrids or not.*** The only way to prevent permanent harm to farmers' export interests is to ban the open-air cultivation of biopharm crops, at the very least food crops.

If biopharming is going to hurt rather than help farmers, what can be done to aid struggling family farmers looking for additional income? This is much too big a topic to address in detail here, but a few proven alternatives can be discussed briefly. Specialty grains such as spelt are growing in popularity and fetch higher prices than traditional grains. The growth in demand for organic grain and produce is projected to be an astronomical 18% per year in the U.S. over the next five years (St. Louis Post-Dispatch 2001). Organic corn and soybean farmers garnered from 35% to more than double the price of conventional crops in the latter half of the 1990s (Welsh 1999). Like biopharming, organic production garners higher returns through emphasizing quality over quantity. Yet because it does not require pollen containment, security measures, precision agriculture equipment and all the rest, most of those higher returns will be returned to the farmer rather than the biopharm company. Organic production also increases rather than decreases a farmer's independence; while certain requirements must be met to go organic, in the long run this production method demands creativity, skill and experience rather than the cookie-cutter farming prescribed by biopharm "specifications." While not possible for all, direct marketing is another proven means for putting a much higher portion of the food dollar in the farmer's pocket.

The USDA must start providing more support for these dynamic and growing alternative production and marketing systems. Very few extension agents are familiar with organic production methods, and USDA research and subsidies for alternative agriculture are a pittance compared to its huge financial commitment to biotechnology and biopharming. For instance, the USDA should follow the European lead and provide assistance to help farmers who wish to go organic get through the 3-year transition period required before their crops can carry the organic label (e.g. MAFF 2000). European initiatives such as this have been taken in response to popular demand for organic foods. As a result, observers predict that 30% of Western European farmland will meet organic production requirements by 2010 (Economist 2000). The USDA should heed the marketplace rather than its biotech industry friends and help farmers provide the kinds of foods that more and more Americans are demanding.

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Appendix 1
Reducing the Uncertainty

Much more complete information on genetically engineered crops is required at the molecular level in order to increase the chances of detecting unintended effects. This information is considered particularly important for future applications that involve engineering the plant with multiple traits (so-called "gene stacking") (Kuiper et al 2001, p. 523). One example of gene stacking is the proposal to utilize avidin corn "as a background germplasm for production of other valuable proteins" (Kramer et al 2000, see Appendix 2).

Full molecular characterization data

Because genetic engineering is a random process, foreign genes may be inserted within the plant's natural genes or in other genomic locations where they cause disruptions in cellular metabolism. Such disruptions are sometimes evident in the form of non-viable or debilitated plants, in which case development can be terminated. Other disruptions may have subtle effects that are difficult to detect, or become evident only under conditions of plant stress. As we have seen, several commercially grown, engineered crops exhibit such effects, and their molecular mechanisms have yet to be elucidated (Section 2.2). More complete information on the transgene and its protein product should increase the likelihood of detecting/explaining unintended effects.

At present, companies applying for commercialization of new genetically engineered varieties often do not report to regulatory officials the complete DNA and protein sequences of the engineered gene and its product ***as inserted and produced in the engineered crop.*** Normally, they only submit information on the genetic construct, which can become altered in the process of genetic engineering, resulting in incorporation of a gene fragment or otherwise modified gene in the plant's genome. At least one genetically engineered crop, Monsanto's MON810 (Yieldgard) Bt corn, contains only a fragment of the full-length transgene that was supposed to be inserted. Incorporation of a gene fragment was apparently due to breakage of the genetic construct during the transformation process. Monsanto has also been unable to detect, much less characterize, the protein actually encoded by this gene fragment (Monsanto Corn 1995, pp. 14-15).

The techniques required for such molecular characterization have been available for some years. Regulatory officials must demand that they be applied and proper information supplied.

Determining the site of insertion

The potential impacts of the foreign gene depend not only on its structure and the precise nature of its protein product, but also on where it happens to end up in the plant's genome. The transgene can be inserted within or near an existing plant gene, causing disruption of cellular metabolism; it may also activate "silent" (i.e. inactive) plant genes, stimulating production of a protein not normally found in the plant.

> "Location and characterization of the place(s) of insertion are the most direct approaches to predicting and identifying possible occurrence of (un-)intended effects due to transgene insertion in recipient plant DNA. Data for transgene flanking regions will give leads for further analysis, in the case of a transgene insertion within or in the proximity of an endogenous [i.e. plant] gene." (Kuiper et al 2001, pp. 516-517)

Kuiper et al recommend several currently available techniques for determining the site(s) of insertion and characterization of adjacent regions in the plant's genome. As in the case of molecular characterization, however, most regulatory agencies do not demand insertional position data from registrant companies, despite its potential usefulness.

One common effect of genetic engineering is the scrambling of DNA sequences adjacent to the transgenic insert. Such scrambling occurred with Monsanto's Roundup Ready soybeans, and was only discovered after years of commercial cultivation of the crop on tens of millions of acres (Windels et al 2001). Many unintended effects have been reported for Roundup Ready soybeans, including lower phytoestrogen levels (Lappe et al 1999); depressed root

development, nodulation and nitrogen fixation; lower levels of aromatic amino acids; lower average yields than their conventional counterparts (Benbrook 2001); as well as increased lignin content (Coghlan 1999). While it appears unlikely that DNA scrambling is responsible for these effects in this case, positional effects may have potentially dangerous consequences in other cases, making it imperative to characterize the site(s) of transgene insertion and adjacent regions of plant genomic DNA.

One surprising limitation in this regard is the lack of full genomic maps for most major food crops that have been engineered (except rice). Complete characterization of the host plant's chromosomal and genetic structure should be demanded before any further genetic engineering experiments, particularly those involving drug-growing plants, are permitted to enter the marketplace.

Profiling techniques to detect unintended changes

Complete characterization of the gene, its protein product and site of insertion in the plant genome are far from adequate, however, for a thorough assessment. One should also determine any effects exerted by the foreign transgene/protein on other components and processes of the plant's cellular machinery. Current assessment procedures examine a very limited array of key nutrients and selected anti-nutrients and toxicants for potential changes in levels of expression relative to a non-engineered control plant; such changes may signal unintended effects that require further analysis. With this "targeted approach:"

> "...unexpected changes are merely identified by chance. The targeted approach has severe limitations with respect to unknown anti-nutrients and natural toxins..." (Kuiper et al, p. 516).

This is because the number of compounds evaluated is a small fraction of the cell's full complement of compounds, and their selection is somewhat arbitrary due to limited knowledge concerning which are most likely to be affected. This has led to calls for a "non-targeted" approach utilizing profiling methods (Ibid, p. 516).

Profiling methods currently available or under development include DNA expression analysis, proteomics, two-dimensional gel electrophoresis, and chemical fingerprinting. These techniques – used singly or in combination – permit simultaneous, small-scale, quantitative analysis of a large array of plant components, including messenger RNA, proteins and metabolites. The virtue of this "non-targeted" approach is that it casts a wide net, implicitly acknowledging what genetic engineers often prefer to ignore: that genetic engineering often causes completely unintended effects, making the crude "targeted" analysis of a few cellular components ineffective as a means for detecting them. Kuiper et al urge rapid refinement and application of these profiling techniques to ensure the most complete assessment possible of unintended effects caused by any application of genetic engineering, particularly those involving gene stacking.

Appendix 2
Avidin Corn: Lab Experiment in the Field

Avidin corn was developed jointly by ProdiGene, of College Station, Texas, Pioneer Hi-Bred International and the U.S. Department of Agriculture (USDA) by engineering the gene for chicken egg avidin into corn (Kramer 2000; Hood et al 1997, p. 292). Avidin corn has been grown in field trials since 1993 (NAS 2002, p. 181). Corn-derived avidin supplied by ProdiGene is presently sold as a research chemical by Sigma Chemical Company (product number A8706; see www.sigmaaldrich.com). Because avidin offers protection against many stored-grain insect pests, avidin corn has been proposed for use as food and feed corn, and as an "insect-resistant background host plant germplasm" for production of "other valuable bio-pharmaceutical or industrial proteins" (Kramer 2000), an example of the gene stacking discussed in Appendix 1.

Avidin causes biotin deficiency in insects, mammals and humans

Avidin is a 66 kDa glycoprotein composed of four identical sub-units. It is found naturally in the egg white of bird, reptile and amphibian eggs. Ingestion of avidin is known to kill or chronically impair twenty-six species of insects (NAS 2002, p. 180). Because avidin deactivates biotin, an essential B vitamin, the mechanism of toxicity is presumed to be biotin-deficiency (Kramer et al 2000, p. 670). Avidin-containing substances such as egg white can also cause biotin deficiency when fed to animals in large quantities, with adverse effects on immune system function, reproduction, prenatal development and growth rate.

Experiments on mice that were fed egg white as part of their diet to induce biotin deficiency showed retarded growth as well as a weakening of the immune system (Baez-Saldana et al 1998, p. 431). The adverse effect of biotin deficiency on immune system function has also been found in rats (Pruzansky & Axelrod 1955; Kumar & Axelrod 1978; Rabin 1983) and guinea pigs (Petrelli et al 1981). In hamsters, biotin deficiency impairs reproductive function and prenatal development (Watanabe 1993). In humans, consumption of large quantities of avidin in the form of raw egg whites is known to cause dermatological, neurologic and ocular disorders (Dorland's Medical Dictionary 1994). Biotin deficiency is also thought to have adverse effects on the human immune system:

> "Fragmented pieces of evidence have suggested that biotin deficiency has a deleterious effect on several immune phenomena, including a higher susceptibility to infections (particularly fungal infections), a diminished antibody response against various antigens, and a decrease in circulating lymphocytes." (Baez-Saldana et al, p. 435).

Has avidin corn already entered the food supply?

Avidin corn is grown on an experimental basis under the nominal authority of the U.S. Department of Agriculture, which is responsible for field trials of genetically engineered plants. An expert committee of the National Academy of Sciences criticized deficiencies in the USDA's regulation of these trials in a recent book, singling out avidin corn as an example (NAS 2002, pp. 180-81; see Section 6). Without proper oversight, avidin corn could contaminate food-grade corn in numerous ways (see Sections 6.4.5 and 7.3.2).

Lapses in gene containment by ProdiGene covered up

Informed sources who wished to remain anonymous detailed several instances in which ProdiGene and farmers it contracted to grow avidin corn failed to follow gene containment protocols designed to prevent escape of the avidin gene. These lapses included failure to "detassel" (cut off the pollen-producing tassel of the corn plant) and failure to clean farm equipment after harvesting avidin corn (necessary to prevent spread of avidin corn seed to conventional fields).

Sterile pollen?

Avidin corn is supposed to have sterile pollen, which is touted as a biological containment measure that prevents escape of the avidin gene. Based on conversations with ProdiGene's John Howard and USDA personnel, the expert NAS committee stated that: "Avidin-producing [corn] plants that express high levels of avidin are male sterile,

presumably due to the toxicity of the avidin to pollen-producing tissues of the corn plant" (NAS 2002, p. 181). In contrast to these personal communications, close examination of the seminal paper on avidin corn by Hood et al (1997) reveals a more ambiguous state of affairs. The only results reported by the authors showed that just 32 of 39 plants (82%) testing positive for the avidin gene were also male sterile; 6 others (15%) had limited fertility, while 1 of 39 (3%) was fertile. Hood et al (1997) lump the first two categories together to obtain their figure of a 97.5% correlation between the avidin gene and "the male sterile/limited fertility phenotypes" (Ibid, Table 2, p. 298). Elsewhere, the authors drop the qualifications "partial" and "limited fertility," giving the impression that avidin corn is completely male sterile.

Given ProdiGene's negligent physical containment practices and the merely ***partial*** male sterility of avidin corn, there have already been many opportunities for the avidin gene to spread to food-grade corn. If it has not occurred yet, it will likely occur in the future as long as continued open-air cultivation of this crop is permitted. Though ProdiGene and USDA personnel have claimed in conversation with NAS committee members that avidin corn is presently being grown on just five acres, there are no publicly available documents to confirm or refute this claim. In any case, as the NAS committee notes in its section on avidin corn, "it would be possible, under notification, to grow thousands of acres of a transgenic crop that produced a substance that was allergenic or toxic to livestock or humans..." (NAS 2002, p. 181).

The dose makes the poison?

Some will argue that even if avidin contaminates food-grade corn, it will not pose a health risk at the levels anticipated in engineered plants. According to Hood et al (1997), however, this transgenic corn produces avidin at "a level higher than any heterologous[19] protein previously reported for maize" (p. 291). Indeed, the 164 parts per million of avidin in corn kernels reported in a recent study (Kramer et al 2000, p. 670) represents 100 to 1,000 times more insecticidal toxin than found in commercial lines of Bt corn[20] (EPA BRAD 2001a, Science Assessment, Table A2).

Would consumption of this much avidin in corn products be dangerous? Kramer (2000) speaks of "egg white injury" resulting from consumption of "several dozen raw eggs a day for several months." To get a rough idea of whether avidin corn might be hazardous to human health, we can compare its avidin content to that of eggs. According to Hood et al (1997), 100 kg of its corn contains over 20 grams of avidin, an amount equivalent to that in 900 kg of eggs (p. 304). With one large egg weighing an average of 50 grams (source: egg carton), each egg contains 1.1 mg avidin. ***100 grams of avidin corn (less than 1/2 lb.), which represents a modest serving of corn grits, contains 20 mg of avidin, or the amount found in 18 eggs.*** [21] While much of the avidin in a highly processed product such as corn flakes would probably be degraded, a substantial percentage would survive intact in minimally processed foods such as corn flour, cornmeal or corn grits. In fact, ProdiGene's experiments show that avidin suffers no loss of activity at and above the standard temperatures used to dry corn (Ibid, p. 300), and that only 18% of the total avidin activity (measured as biotin-binding capacity) is lost during dry milling (Ibid, p. 297). Dry milling is the process used to prepare corn flour, corn grits and similar products from whole corn.

Thus, it is no wonder that even proponents of avidin-corn admit that: "Long-term ingestion of high levels of avidin maize may be a problem, because a biotin deficiency can decrease the growth rate of mice and affect reproduction." Of course, solutions are always available:

> "...avidin has an antidote (biotin), which can be used to prevent toxicity or to rescue potential victims from adverse effects. Food and feed uses of avidin maize might involve processing that includes supplementation with the vitamin" (Kramer et al 2000, p. 672).

It is probably safe to assume that most "potential victims" would prefer that the toxin be kept out of the food supply in the first place rather than depend on the food processing industry to "rescue" them from adverse effects through adding an "antidote."

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[19] In this context, "heterologous" means "foreign," in the sense of a protein not naturally produced in corn.

[20] The insecticidal Cry1Ab protein spliced into two commercialized Bt corn "events" is present in kernels at levels of 0.2-0.4 ppm (Monsanto's MON810) and 1.4 ppm (Syngenta's Bt11).

[21] Avidin content ranges from 15-30 grams per 100 kg grain for avidin corn from different generations or grown in different locations, so 20 grams is a conservative estimate. For corn plants with 30 g/100 kg, 100 grams of avidin corn contains the equivalent of 27 eggs, over the "couple of dozen" cited by Kramer as a human health hazard.

Unintended consequences

Such a "solution" to an engineered problem would not be justified even if avidin corn were otherwise a thoroughly safe and well-characterized crop. In fact, however, scientists have detected a number of totally unexpected – and yet unexplained – effects of engineering the avidin gene into corn.

1) Male sterility

As noted above, most avidin-producing plants appear to be partially or completely male sterile (Hood et al 1997, p. 297), an effect that "may be the result of the avidin protein affecting the activity of a molecule essential for pollen development" (Ibid, p. 304). This speculation is little more than a restatement of the observed effect, however, not an explanation of the phenomenon.

2) Kernels vary greatly in avidin content

Corn plants have both male and female sexual organs, and thus can either self- or cross-pollinate. The partial male sterility of avidin corn, however, mostly precludes self-pollination and necessitates outcrossing to non-avidin corn. Due to the nature of corn genetics as presently understood: "In theory, 50% of the kernels should not contain avidin, since the avidin-expressing plants were male sterile." Yet instead of the clean results predicted by theory, testing on individual kernels revealed that "avidin concentration was highly variable, with levels ranging from 0-2,500 ppm" (Kramer et al 2000, p. 670). In other words, the first unintended effect (male sterility) did not produce its expected consequence (zero avidin in half the kernels), and scientists do not have any explanation for either the primary or secondary unintended effects.

3) Two-fold difference in avidin content

There was also a two-fold difference in avidin content of large populations of transgenic corn from different generations grown in the same location, as well as from corn of the same generation grown in different locations. Once again, there is no explanation of these phenomena, beyond a generic reference to nature-nurture interactions: "Though the cause of this variation is unknown, the interaction of genotype with environment is well documented" (Hood et al 1997, p. 304).

4) Loss of herbicide resistance

Avidin corn was originally engineered with a *bar* selectable marker gene (4 copies) for herbicide resistance for the purpose of selecting those plant cells that had successfully incorporated the avidin gene (3-5 copies). However: "The avidin-expressing line lost resistance in the T1 generation to the herbicide on which it was originally selected in culture" (Ibid, p. 298). There is no explanation for this loss of herbicide resistance, which is particularly puzzling given the following assertion, which was also made with respect to plants of the T1 generation: "The avidin and bar inserts appear to be inherited as a single linkage unit...." If in fact "the linked avidin and bar genes were segregating as a single unit among individuals in these populations...," it is difficult to understand how plants could lose herbicide resistance without also losing the avidin gene, since a "single linkage unit" by definition rarely becomes separated (Ibid, p. 297).

5) Other pleiotropic effects: female sterility and toxicity

Other transformation events exhibited other unintended effects, including female sterility and toxicity to the plant itself. Female sterility was unexplained, while the latter effect was attributed to intracellular accumulation of avidin due to lack of a signal telling the cell to secrete the protein into the intercellular compartment, where its toxicity apparently does not harm the cell (except presumably in pollen). These effects were apparently not observed in the avidin-producing lines chosen for further development, but they raise an important issue.

In order to express foreign proteins in a plant, the transgene must first be attached to a sequence of DNA known as a promoter. Promoters are like on switches that instruct the cell to generate the protein encoded by the attached transgene. Most promoters used in genetic engineering today are "constitutive" – that is, they are able to "turn on" the foreign gene in all cellular tissues, resulting in production of the foreign protein in all parts of the plant: seed, leaves, stem, roots, pollen, etc. In contrast, "tissue-preferred" promoters are supposed to yield higher levels of protein in a single part of the plant, but often produce lower levels in other plant tissues (Aprotinin Patent 1998; see also Section 5.6.3).

Constitutive promoters are generally expected to elicit unintended effects because the ubiquitous presence of the foreign protein gives it more opportunities to disrupt the development and functioning of various tissues.
Because avidin-producing corn utilizes such a constitutive promoter, "one might predict that other physiological phenotypes would be encountered" (Ibid, p. 304). However, the nature of these unintended effects – male and female sterility, for example – was completely unpredictable and has yet to be explained.

Comparison of corn-produced and native chicken-egg avidin

Corn-derived avidin was compared to its native chicken egg counterpart using a number of tests. First of all, the 25 amino acids at the N-terminal of each protein were sequenced, and proved to be identical. Secondly, the molecular weights of the two proteins were shown to be different. Corn-derived avidin weighed 5% less than the native chicken egg version, a difference that was attributed to smaller carbohydrate groups attached to the corn version, since deglycosylation of the two different avidins yielded proteins that appear to be the same size. According to Hood et al: "These [glycosylation] data, combined with the N-terminal sequence data (Table 5) strongly suggest that the primary sequence of the two avidins is identical" (Ibid, p. 302). Yet the avidin sub-unit (4 identical sub-units make up the 66 kDa molecule) is 152 amino acids long. Identity of a mere 16% (25/152) of the amino acids in the two proteins is obviously no proof that the primary structure of the two avidins is identical, even if the deglycosylated molecular weights do appear to coincide. There is no substitute for full sequencing of the corn-grown avidin, both to detect any potentially significant amino acid differences vis-à-vis native avidin and to establish the true molecular weight.

Could corn-derived avidin be allergenic?

Native avidin from egg-white and a related compound, streptavidin, are known to cause immune responses in humans (Subramanian & Adiga 1997, Meyer et al 2001), though they apparently have no history of causing allergies (Langeland 1983). What about corn-grown avidin? As noted above, ProdiGene scientists failed to compare the full primary structures of the two avidins, but did find a clear difference in the size of the carbohydrate groups attached to corn (4.3 kDa) versus chicken-egg (5.1 kDa) avidin sub-units. The successful removal of the carbohydrate groups of both versions of avidin by N-glycosidase A (Hood et al 1997, p. 302) indicates the presence of the N-linked carbohydrate groups most associated with allergy (SAP MT 2000, p. 23). The immunogenicity of native avidin, the N-linkage of the carbohydrate groups, the altered glycosylation pattern of corn avidin, and the high expression level of the protein all raise serious allergy concerns that require further investigation.

Avidin to be engineered into corn together with pharmaceuticals?

Despite the known risks and unexplained effects discussed above, there is already talk of stacking avidin corn with other genes, compounding one poorly characterized genetic experiment with a series of others. In particular, it is being proposed as an "insect-resistant background host plant germplasm" for production of "other valuable bio-pharmaceutical or industrial proteins" in corn (Kramer 2000). It is hoped that the presence of avidin would protect the corn grain and its co-engineered protein(s) against pest infestation and degradation, providing by far the cheapest storage option for the engineered protein – grain silos (ProdiGene Benefits 1999). Since avidin is intended to deter mainly stored grain insects, however, stacking of a third gene for field pest resistance (such as a Bt protein) is entirely conceivable. Each combination would require extremely careful testing for unintended effects caused by each gene insertion, separately and in combination. As discussed in Appendix 3, two insecticides being considered for splicing into a single crop exhibit synergistic effects. Stacking drug genes into avidin corn would also then necessitate additional purification steps to remove the pesticide from the drug, as discussed in Section 4.2.3.

Avidin corn: industry and government neglect public safety

The USDA jointly developed avidin corn with ProdiGene, and has promoted this crop in its popular literature (USDA Avidin 2000, an article entitled "Avidin: An Egg-Citing Insecticidal Protein in Corn"). Thus, it is not surprising that the agency overlooked ProdiGene's sloppy genetic containment practices and squelched a section of the NAS report that detailed them. Given the likelihood of contamination, the many proven and potential adverse impacts of avidin, and ProdiGene's failure to explain numerous unintended effects or even properly characterize its corn-grown avidin, avidin corn should no longer be grown in the open air.

Appendix 3
Health and Environmental Risks of Aprotinin and Protease Inhibitors

Aprotinin corn was developed by scientists with ProdiGene, Pioneer Hi-Bred International, Eli Lilly & Company and PE Applied Biosystems by inserting a modified gene sequence for cow aprotinin into corn (Zhong et al 1999).

Medical uses of aprotinin

Aprotinin is a protease inhibitor – a substance that inhibits the action of protein-degrading enzymes – that has uses in biochemical research, medicine, and potentially in agriculture. It has traditionally been extracted from bovine lung tissue, and is sold by Bayer under the name of Trasylol. It is best known as a clotting agent used to reduce blood loss in heart surgery (Landis et al 2001), and has also been administered for over three decades in the treatment of acute pancreatitis (Belorgey et al 1996, p. 555). Aprotinin's coagulant activity has led to recommendations that it not be used on normally clotting patients due to the risk of thrombosis (blood clot) (Blomgart et al), though more recently this risk has been discounted (Landis et al 2001). In rare first-use cases, aprotinin has caused life-threatening anaphylactic reactions, a risk that increases significantly (up to 5% of cases) upon re-exposure (Trasylol Label 1999). Since aprotinin is infused intravenously for these medical applications, it probably does not pose the same risks when ingested, inhaled or through skin contact, though studies of these latter routes of exposure appear to be lacking.

Has the food supply been contaminated with aprotinin?

Aprotinin corn has been grown at least since 1998, when it was reportedly cultivated in field trials by farmers under contract with ProdiGene's partner, Stauffer Seeds, in Hamilton County, Nebraska (Seed and Crops Digest 1998). However, the USDA biotech website does not identify a field trial of aprotinin corn until 2002 in Hawaii (APHIS Permit No. 01-187-01r). This indicates that the identity of the aprotinin gene was kept secret as confidential business information in the listings for the 1998 and any previous or subsequent trials (see Section 6.3.1) until 2002. The only reported biopharm corn field trials conducted by ProdiGene in Nebraska in 1997 and 1998 are listed under APHIS Permit Nos. 97-098-07n and 98-085-42n, on 5 and 4 acres, respectively. While the identity of the gene is kept secret, the gene category is "novel protein" in each case, while the 2002 trial of aprotinin is listed under the "pharmaceutical protein" category. The USDA must have reclassified aprotinin from novel to pharmaceutical protein, otherwise there is no accounting for the independently reported 1998 field trial of aprotinin in Hamilton Country, Nebraska.

It is possible that aprotinin corn has been cultivated in strict isolation from normal corn, though the secrecy of the USDA and ProdiGene make it impossible to determine this. If aprotinin corn was grown according to USDA performance standards for "minimization" of gene flow, which recommend isolation distances of either 660 feet or 1320 feet (Section 6.4.5) from other corn, it is possible that food-grade corn has been contaminated. This becomes more likely when we consider that the company is ProdiGene, which was has been observed to be negligent in gene containment practices with its avidin corn, and that aprotinin corn is not reported to be even partially male sterile, increasing the likelihood of contamination through cross-pollination.

Allergenic potential

As noted above, aprotinin has been found to cause anaphylaxis, a life-threatening allergic reaction, especially upon repeated intravenous uses. Aprotinin is a fairly stable molecule that resists degradation by enzymes and acids, and also has significant thermal stability (Sigma Aprotinin). Since these are common properties of food allergens, aprotinin should be properly evaluated for possible allergenic effects from ingestion, inhalation and dermal contact – especially in its corn-grown form, which apparently has not been tested for glycosylation (Zhong et al 1999, p. 353).[22] As discussed in Section 4.1.1, plant glycosylation patterns would also heighten allergy concerns.

[22] Zhong et al say only that: "There was no evidence to support a protein being glycosylated, nor is it glycosylated in its native form from eggs." Examination of the paper shows no indication that any specific tests for glycosylation were carried out. The

Pancreatic disease from ingestion of protease inhibitors

Aprotinin presents other, potentially more serious, health concerns as a protease inhibitor. Protease inhibitors are found naturally in legumes and particularly in soybeans, and are known to be toxic to many insects, fungi and animals. Animal feeding studies have shown that these inhibitors depress growth by interfering with the digestive activity of enzymes like trypsin that are secreted by the pancreas. This inhibitory effect on trypsin causes the pancreas to compensate by secreting more trypsin-containing digestive fluids, resulting in abnormal enlargement of the organ's cells (hypertrophy) and abnormal increase in number of cells (hyperplasia). Prolonged feeding of soybean trypsin inhibitors leads to development of tumorous nodules on the pancreas, which after 60 or more weeks become cancerous (SAP MT 2000, pp. 31-33).

Whether ingestion of protease inhibitors is similarly dangerous to humans is not certain, though there is evidence that aprotinin (Dlugosz et al 1988) and other protease inhibitors (SAP MT 2000, p. 31) do in fact stimulate secretion of trypsin and other digestive enzymes in humans as in animals. "This would indicate that the human pancreas at least responds in a negative fashion to the effects of a protease inhibitor" (Ibid, p. 31). Additional evidence of human impacts is the report of an outbreak of gastrointestinal illness in individuals who had consumed under-processed soy protein extender in tuna fish salad. This outbreak was attributed to the protease inhibitors in the soy protein, which apparently had not been deactivated by the usual heat treatment used in soybean processing (Ibid). This case suggests that a relatively small quantity of protease inhibitors (that present in a soy protein ***additive***) may be sufficient to cause symptoms, at least in certain individuals.

Other potential human health risks of protease inhibitors

It is interesting to note that scientists still do not understand how protease inhibitors kill insects. Some attribute this effect directly to inhibition of digestive enzymes in the insect gut (SAP MT 2000, p. 31), also the presumed mechanism of gastrointestinal illness in higher animals. But others disagree, proposing different mechanisms.

> "The mechanism of action of proteinase inhibitors is not fully understood. Inhibition of enzymes in the alimentary tract of insects is not the main adverse effect. ***Depletion of essential amino acids due to over-secretion of digestive enzymes in the presence of inhibitors*** is thought to cause most of the toxicity signs observed, but ***there are also other targets of toxicity***." (Kleter et al 2000, section 2.2.3, emphasis added)

Thus, in both insects and mammals, it appears that protease inhibitors: 1) Inhibit digestive enzymes; and 2) Thereby stimulate over-secretion of these same enzymes in a negative feedback loop. In mammals, this leads to pancreatic disease. In insects, it triggers a third effect – depletion of essential amino acids – which some suggest is the chief mechanism of toxicity. Do protease inhibitors have this latter effect in humans as well? If so, depletion of essential amino acids could present the risk of nutritional deficiency. And what of the "other targets of toxicity"? Could they too have human analogues?

Aprotinin and other protease inhibitors as plant pesticides

Because of their insecticidal activity, protease inhibitors like aprotinin are being experimentally spliced into crops to protect against insect attack. ProdiGene is clearly interested in this insecticidal application, as indicated by a passage from its patent for "Commercial production of aprotinin in plants" (Aprotinin Patent 1998):

> "Fortuitously, it has been determined that the serine-specific proteinase inhibitor aprotinin has potent insecticidal or larvicidal activity when administered enterically to insects such as European corn borer (ECB) and corn rootworm."

ProdiGene has shown that aprotinin causes 25% mortality in European corn borer larvae after 7 days of feeding with just 1.0 mg aprotinin per ml of feed. Corn rootworm, the only other insect tested, experienced 60% mortality with 20 mg aprotinin per ml of feed over 7 days (Aprotinin Patent 1998, Tables 3 & 4).

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reference to "native" aprotinin from eggs is puzzling, since the aprotinin gene spliced into corn is reverse translated from the bovine protein, and aprotinin derived from bovine tissue would thus seem to be the proper comparator. In addition, bovine lung is by far the most common source of aprotinin for research and medical purposes, and I find no other reference to aprotinin from egg.

Aprotinin shortens the lives of honeybees

Could aprotinin in plants harm non-target insects? Several feeding experiments have shown that the lives of honeybees are significantly shortened when they consume as little as 3-18 μg aprotinin per day for seven days (Malone et al 2001, p. 64; Burgess et al 1996). The daily dose of 18 μg resulted from feeding honeybees pollen-food containing aprotinin at a concentration of 2.5 mg/g; this concentration was chosen to simulate exposure to transgenic pollen expressing 1% aprotinin (of total protein), approximating a credible field situation (Malone et al 2001, p. 64-5). Unfortunately, ProdiGene does not report the level of aprotinin in the pollen of its corn. Yet its use of the constitutive ubiquitin promoter suggests that expression in pollen is possible. This possibility becomes more likely when one considers that Cry1F corn, which also contains an ubiquitin promoter, expresses Cry1F in pollen (EPA BRAD 2001b, p. 7). As of 1999, ProdiGene had achieved expression levels of aprotinin in corn kernels averaging about 0.1%, but with some plants expressing up to 0.44%, of total soluble protein; new production lines were to be generated from these higher-expressing plants (Zhong et al 1999, p. 352). The aprotinin content of pollen and anther should be measured and appropriate studies done to determine any possible impacts on a wide range of non-target insects.

Stacking aprotinin with other insecticides

An emerging strategy in the biotechnology industry involves engineering several insecticides into a single crop to achieve broader-spectrum and/or more potent insecticidal activity.

> "Combining proteinase inhibitors with lectins or with Cry proteins, either by cross breeding of primary transformants or by multiple gene insertion, is also contemplated in order to enhance insect resistance." (Kleter et al 2000, 2.2.4).

ProdiGene is clearly interested in this strategy:

> "Furthermore, aprotinin and highly similar serine proteinase inhibitors strongly potentiate the insecticidal activity of lectins such as wheat germ agglutinin. It appears that a transgenic plant expressing aprotinin would potentially be more resistant to plant pests such as ECB and corn rootworm" (Aprotinin Patent 1998).

1.0 mg aprotinin per ml of feed combined with 0.2 mg wheat germ agglutinin (WGA) per ml of feed causes 80% mortality in ECB larvae, exhibiting a powerful synergistic effect (Aprotinin Patent 1998, Table 4). Thus, stacking aprotinin and other protease inhibitors with other pesticides could have significant impacts on non-target insects such as honeybees.

Conclusion

Scientific advisors to the EPA recommend that transgenic plants expressing protease inhibitors and/or lectins be subjected to animal feeding studies (SAP MT 2000, p. 33-34). Attempts to discover whether the USDA or FDA had conducted any tests to gauge the potential health risks of aprotinin were unsuccessful. The USDA's limited response to a Friends of the Earth Freedom of Information Act request contained nothing concerning aprotinin. An FDA scientist said that while the FDA might consult with the USDA on certain biopharm plantings, she was not at liberty to discuss aprotinin or any particular product (personal communication, 2/8/02, Kathryn Stein, formerly of FDA).

Appendix 4
A Case Study of Virally-Vectored Trichosanthin in Tobacco

Another way to grow drugs in plants involves infecting them with genetically engineered viruses. The desired gene is first inserted into the virus, which is then used as a vector to infect the plant with the biopharm gene. The plant is thus forced to produce the biopharmaceutical along with the virus's own proteins, in the process becoming diseased. The drug is then extracted from the plant tissue.

Plant viruses – still many unknowns

One concern is that genetically engineered viral vectors used to infect plants could "cross over" and infect animals or humans. One study has found evidence that at some time in the past, a plant nanovirus crossed over to infect a vertebrate, possibly through exposure to sap from the infected plant. It then recombined with a vertebrate-infecting calicivirus (of the same family as the rabbit virus discussed in the Section 4.7) (Gibbs & Weiller 1999). While the plant viruses used thus far in open-air biopharming experiments seem to be unable to infect animal tissue, others can replicate in both plants and animals:

> "...we have to recognize that there are some viruses, hopefully none that are going to be used for vectoring purposes, that have the ability to replicate in plant or in animal tissue... We know, for instance, of [examples] even among the rhabdoviruses, and there are other viral families that have these abilities..." (Dr. Charles Rupprecht, Biologics Meeting I 2000, p. 128)

Rupprecht, an expert on viruses with the Centers for Disease Control, points out the common fallacy of genetic engineers who maintain that lack of evidence of health or environmental impacts of their activities is a reasonable demonstration of their safety – the "don't look, don't find" mentality:

> "...we have no documentation that plant biologics or plant viruses have any notable clinical effects on people or other mammals, but one has to ask the question, and to raise the issue, of how hard one has looked. From a virological standpoint, there are many, many more unclassified viruses than classified, and many, many more uncharacterized viruses than those few that we deal with..." (Ibid, p. 126)

In answer to the self-posed question – "[c]ould plant viruses be involved in any clinical conditions, be they human or other animal?" – Rupprecht's reply bears careful consideration:

> "In fact, it wouldn't be too difficult to predict that now that we've got the tools available and given the realms of the majority of uncharacterized plant viruses that sooner or later somebody will put that connection together. It just hasn't been done yet." (Ibid, p. 126)

Case study of trichosanthin-producing tobacco

Since 1991, there have been at least ten open-air experiments in which genetically engineered viruses were used as vectors to infect tobacco with biopharmaceutical genes. Many more such trials are presumably being conducted in greenhouses. In eight of the ten trials, the USDA kept the identity of the engineered drug genes secret as "confidential business information" of the applicant. The other two trials involved trichosanthin, a drug derived from the roots of a Chinese plant.

Trichosanthin belongs to the class of ribosomal inhibitor proteins (RIPs), which operate by inactivating a cell's protein-making machinery (e.g. ribosomes). Trichosanthin is similar to two other members of this group – ricin and abrin – that are among the most toxic substances known to man. It is an extremely potent RIP, able to inhibit protein synthesis by 50% in an assay involving young rabbit blood cells at a concentration of just 0.1 ng/ml (Kumagai et al 1993, p. 430). Trichosanthin has a long history of use in China to induce abortions. Effects associated with the intravenous use of trichosanthin include toxicity to embryos and fetuses (Chan et al 1993), renal toxicity (Ko & Tam 1994), neurological disorders (Kahn et al 1990), fever, headache, arthralgia and skin rashes

(Dharmananda, S.). In these two trials, which were conducted by Biosource Genetics in 1991 (North Carolina) and 1996 (Kentucky), the gene for trichosanthin was engineered into tobacco mosaic virus (TMV), which was used as a vector to infect tobacco plants and force them to express the drug.

Tobacco mosaic virus (TMV)

TMV is the most commonly used viral vector for these genetic experiments, accounting for 9 of the 10 open-air biopharm field trials conducted thus far. TMV has been called "one of the most well-known viruses on the planet" (Barry Holtz of Large Scale Biology, Biologics Meeting I 2000, p. 61). Yet virus expert Dr. Allen Miller admits that "even with TMV, there's a lot of work to be done to really understand what's going on that would help optimize viral general expression" (Ibid, p. 32). An example of what we have yet to learn about this "most well-known virus on the planet" is the function of several major proteins encoded by TMV genes. "The 126K and 183K proteins are ***presumably*** involved in the replication of RNA (Palukaitus and Zaitlin, 1986), ***the function of the 54K polypeptide is unknown*** (Sulzinski et al, 1985)..." (USDA EA 91-007-08, 1991, p. 11, my emphasis).

TMV belongs to the tobamovirus group, which consists of 50 families of viruses found around the world. Examples include the type strain tobacco mosaic virus, tomato mosaic virus, sunn-hemp mosaic virus and cucumber green mottle virus. The most closely related members of the group are the TMV type strain and tomato mosaic virus (ToMV), which have an extremely high 85% genetic similarity (Ibid, p. 10). Most agricultural experts treat TMV and ToMV as one because of their genetic similarity, common host range, and the indistinguishable symptoms they cause in infected plants. Besides tobacco, the crop plants susceptible to infection by TMV/ToMV strains include tomato, eggplant, pepper and potato. Weeds and other plants that can harbor the virus include nightshade, amaranth, goosefoot, petunia, horsenettle, jimsonweed, Jerusalem cherry, ground cherry and plantain. TMV is also seen on apple, beet, sugarbeet, buckwheat, currant, grape, pear, spinach and turnip (U of CT IPM 1998; NCSU PP).

TMV is easily spread

TMV is transmitted in numerous ways: by touch, by plant debris carried on workers' clothing, or in tobacco products. The latter route of transmission explains why tomato pickers are often prohibited from carrying or using tobacco products, where the virus can survive for many years. Contaminated farm implements and animals can also spread TMV. The ability of TMV to survive the winter in many common weeds, as well as in tobacco seeds and in the soil, means that unexpected infections can turn up the next growing season (U of CT IPM 1998; NCSU PP).

Containment of TMV

Containment measures include frequent hand washing, cleaning farm equipment with bleach, treatment of seeds with heat or trisodium phosphate, and steam treatment of infected soil. But the only really sure means of stopping TMV in an infected field is to plant non-susceptible species for two years (U of CT IPM 1998; NCSU PP).

Serious inadequacies in the USDA's environmental assessment

The following discussion is based primarily on an environmental assessment conducted by the USDA prior to granting the first field trial permit noted above for production of trichosanthin in tobacco (USDA EA 91-007-08, 1991; unless otherwise noted, references are to this EA). The trial took place near Raleigh, North Carolina in 1991.

1) Short EA: The EA is extremely short (only 17 pages of text), especially considering the fact that this was the first open-air release of a virus engineered with a biopharm gene.

2) Oversight delegated to Biosource: The registrant company, Biosource Genetics, was given almost complete responsibility for oversight of the trial, with only one visit provided for by a USDA representative "at the initiation of the experiment or shortly thereafter to verify information about the test protocol" (p. 16). Thus, the USDA was apparently not involved in monitoring for possible spread of the virus beyond the test plot, analysis of trichosanthin accumulation in the tobacco, etc.

3) Could trichosanthin-containing TMV spread to food crops? Two crucial assumptions in the EA are rendered questionable by the expert advice of local agricultural experts:

a) The risk of the engineered TMV spreading to other crops is discounted on extremely thin evidence. The USDA cites just one study (Brunt 1986) to back the assertion that "TMV rarely occurs in tomato crops worldwide" (p. 10). As opposed to this "worldwide" assertion, the North Carolina State University Plant Pathology Extension Service states baldly that: "The most important virus disease on tomatoes in North Carolina is tobacco mosaic virus (TMV) ... There are many strains of TMV and a few which occur in North Carolina are very damaging" (NCSU PP).

b) Dr. G. V. Gooding is cited in a personal communication as claiming that workers' clothes and smoking products "...are completely insignificant..." as sources of TMV inoculum in North Carolina (p. 10). Yet both the N.C. State University Plant Pathology Extension Service and the University of Connecticut Integrated Pest Management Service warn against the risk of TMV spreading to tomatoes through contact with tobacco products. To prevent spread of TMV, NCSU PP recommends that those handling tomatoes "avoid tobacco products and plants"; U of CT (1998) advises workers not to "carry or use tobacco products near the plants," to wash their hands well after tobacco use, and to ensure that their "clothing [is] not contaminated with tomato, tobacco or other host-plant material." (U of CT 1998).

4) Will TMV engineered with trichosanthin survive in the environment? One of USDA's main arguments against the potential for propagation of the genetically engineered TMV virus was the supposition that engineered viruses are generally "outcompeted" by native non-engineered varieties, or on the other hand "revert quickly to their non-engineered counterparts" (p. 14). The USDA fails to supply any hard evidence on the TMV viruses engineered with the genes in question to support this supposition, despite the allusion to prior greenhouse experiments, where one would expect such data to have been collected. Instead, the agency relies on a handful of studies concerning TMV and other viruses engineered with genes other than the trichosanthin gene. As noted above, TMV has been found in many food crops and weeds.

5) USDA incorrectly assumes low level of expression and toxicity to plant (pp. 13-14)

a) The agency assumed that the level of trichosanthin in the infected tobacco "should be below any significant level of biological activity," basing this assumption ***not*** on greenhouse experiments with virally-vectored trichosanthin, but rather on a single prior experiment in which tobacco infected with TMV that had been engineered with a completely different gene was found to contain minimal levels of the gene's product (less than 50 ng chloramphenicol acetyltransferase per gram fresh tissue).

b) The agency assumed that tobacco plants would die if high levels of trichosanthin were generated, thus containing the engineered virus in the dying plant. While we do not know how much trichosanthin was generated in this experiment, a paper published just 1 1/2 years later reported that TMV-vectored trichosanthin was generated at a level of 2% of total soluble protein in tobacco, ***"the highest accumulation of a foreign protein ever reported in any genetically engineered plant"*** (Kumagai et al 1993, p. 429, my emphasis). This level is clearly well beyond what the USDA had assumed, on the basis of little or no evidence, was possible. Contrary to the USDA's assumption, there was no indication that the tobacco plants were killed by this extremely high level of trichosanthin: "The viral symptoms consisted of plant stunting with mild chlorosis and distortion of systemic leaves..." (Ibid, p. 429).

6) Potential human health impacts: Could dermal, inhalant or oral exposure to this level of trichosanthin endanger human health? We don't know. The USDA's EA says only that:

> "Human therapeutic uses of trichosanthin requires [sic] systemic introduction and no successful oral routes of introduction have been noted (Dr. Michael Piatek, personal communication)" (p. 12).

Rather than rely on a personal communication with a single doctor, the USDA might have taken the trouble to consult published studies on the many human health impacts of this powerful drug (cited above), or at least consult with the FDA. At the time of the field trial, trichosanthin was being hailed as the next big AIDS drug, spurring clinical studies as well as unofficial uses. As early as 1989, two years before this field trial, the FDA had issued an alert for automatic detention of trichosanthin being imported from China due to "significant safety concerns." These concerns were based on reports of serious adverse reactions and allegations of deaths related to an AIDS "treatment" being promoted by an AIDS activist group in San Francisco, Project Inform (FDA

Detention 1989). The FDA also noted that in China, trichosanthin is restricted to one administration per lifetime to induce abortions. While trichosanthin is injected for these purposes, Health Canada was concerned enough about ***oral*** use of the drug that it recently issued a warning against ingestion of a Chinese medication containing "trichosanthin alkaloid, ***which is known to cause mutations in human cells and malformations in embryos, suppress the immune system, and produce severe allergic reactions. The safe and effective dose of this herb is not known.***" (Health Canada 2001).

The one-use rule cited above by the FDA for inducing abortions might have to do with the drug's strong allergenicity; trichosanthin can cause anaphylactic reactions, and its initial promise as an AIDS drug in 1990 was undermined by its strong immunogenicity upon prolonged use (Dharmananda, S.). Yet both sensitization and reaction to an allergen can occur upon inhalation, contact or ingestion, three routes of exposure that the USDA does not investigate in its cursory EA, even though they are likely avenues for trichosanthin to be absorbed by workers who harvest and process the tobacco plants. Inhalant allergens, in particular, can act at very small doses.

Repeat trials may indicate continued experimentation with trichosanthin-producing tobacco

The USDA cites only two field trials with trichosanthin on its website. The trial discussed above took place in 1991 in North Carolina (Permit No. 91-007-08r). The same company conducted a repeat trial of trichosanthin-tobacco in 1996, this time in Kentucky (Permit No. 96-051-04r). Repeat trials generally indicate continued interest in commercial development, and often involve larger areas than initial pilot trials. The acreage of this 1996 experiment is not disclosed, nor is an environmental assessment available. But we do know that the same company (Biosource) used the same TMV-tobacco system in three field trials it conducted in the same state as the 1996 trichosanthin trial (Kentucky). These trials were conducted in 1998 (30 acres), 1999 (32 acres) and 2000 (acreage withheld)[23]. Because the genes for these trials were kept secret as confidential business information, we have no way of knowing whether they involved trichosanthin, though they are listed as "pharmaceutical proteins." If they do, the large size of the first two trials would greatly increase the risk of trichosanthin-bearing virus escaping into the environment, as well as the risk of health impacts on those involved in harvesting and processing the host tobacco plants.

[23] Permit Nos. 98-061-01r, 99-048-03r and 00-049-01r, respectively.

Appendix 5
Alternatives to Open-Air Biopharming

Proven Techniques

Extraction from Animal/Human Tissues

Biopharmaceuticals are traditionally obtained from human or animal tissues. For instance, human serum albumin is extracted from donated blood, the growth hormone erythropoietin is isolated from human urine, and insulin is derived from cow and pig pancreas. Some diabetics who respond well to animal-derived insulin can have dangerous reactions to engineered versions of human insulin produced in bacteria or yeast (The Globe and Mail 2002).

Bacterial, Yeast and Animal Cell Cultures

A newer technique involves engineering the appropriate gene into animal cells, yeast or bacteria, which are used as micro-factories to generate the desired substance in large fermentation tanks. Erythropoietin, for instance, is now more commonly obtained from cultures of Chinese hamster ovary cells, a production system used for many biopharmaceuticals. One form of hepatitis B vaccine is produced in yeast, while granulocyte macrophage colony stimulating factor and interleukin-4 are produced in bacteria. These methods are the most commercially developed means of producing biopharm proteins, as evidenced by the size of the industry. As of the year 2000, the worldwide fermentation capacity for production of biopharmaceuticals was approximately 400,000 liters, with products valued at $800 million (Biopharm Backgrounder 2000).

These methods have several clear advantages over open-air biopharming in plants.

1) Proven versus experimental: They are proven techniques with a track record of safe, marketable products, while biopharming is an experimental method that has not produced any major commercial products and is dependent on venture capital and government subsidies.

2) Purification simpler: Animal cells, bacteria and yeast generally contain a much smaller number of extraneous compounds than whole plants, which can be composed of thousands of substances (e.g. the tobacco plant has roughly 4,000 constituents). Thus, purification of the biopharm protein from the plant-tissue matrix can pose considerably greater challenges.

3) Allergic reactions to plant-specific sugar groups: Animal cell cultures process biopharm proteins in ways more familiar to the human system than plants. The plant-specific sugar groups that plants attach to proteins (in a process known as glycosylation) are more likely to elicit allergic reactions than the mammalian glycosylation pattern of proteins generated in animal cell cultures.

4) Controlled and contained: Fermentation takes place in factories under controlled and contained conditions, while biopharm plants are subject to the many vagaries of the natural environment. Controlled conditions favor consistent drug quality and yield; physical containment reduces the risk of contaminating the environment with biopharm traits. Theft and illicit use are much less likely in a factory situation.

Drawbacks of these fermentation methods include the need to screen for animal viruses (animal cell production only) and the relatively high cost of facilities for fermentation tank production.

Alternative Plant-Based Techniques

75% of the world's population relies on plants for treating illness/disease, and nearly 25% of the U.S. pharmaceutical market is made up of plant-derived compounds (DeFuria 1996). Thus, it is natural for researchers to turn to plant systems to meet the projected increase in demand for biopharmaceuticals in the coming years.

Plant Cell Cultures

Culturing plant cells to grow biopharm proteins offers what is in many ways a superior alternative to open-air biopharming. The leading example is the anti-cancer drug Taxol, which is used in the treatment of breast, ovarian and non-small lung cancer, as well as the AIDS-related Kaposi's sarcoma. Taxol was first derived from the bark and then the leaves of the yew tree. Bristol-Meyer Squibb and a German company known as Phyton have developed a method to produce this substance in yew cell cultures. Mitsui Petrochemical Company uses plant-cell cultures to produce commercial quantities of the antibacterial agents berberine and shikonin as well as the nutraceutical ginseng (Francis, A. 2000).

Some of the same substances produced experimentally in plants have also been generated in plant cell cultures. A hormone that promotes growth of white-blood cells, known as granulocyte-macrophage colony stimulating factor (GM-CSF), has been grown in both tobacco plants (Giddings et al 2000, p. 1154) and tobacco cell culture (Lee et al 1997; James et al 2000). Human alpha-1-antitrypsin (AAT), which is used to treat a rare form of emphysema (Merck Manual 1992, p. 666) and cystic fibrosis, has been experimentally produced in both transgenic rice (Amberwaves 2001) and rice cell cultures (Terashima et al 1999). Applied Phytologics has grown AAT using both methods – in open-air field trials of transgenic rice and under controlled conditions in rice cell cultures (Huang et al 2001). In the latter case, the authors report very promising results: AAT was generated at extremely high levels (20% of total secreted proteins) and with a purity of greater than 95%.

Another example is the abortion-inducing drug trichosanthin. It has been grown experimentally out of doors in virally-vectored tobacco (Appendix 4) as well as in plant cell cultures (Stoner et al 1997; see also www.engr.ucdavis.edu/~pse/karen/research.htm) and bacteria (Shaw et al 1991). Peterson & Alfermann (2001) have experimentally produced cancer chemotherapy drugs (cytotoxic lignans) in plant cell cultures, while Magnuson et al (1998) have generated interleukin-2 and interleukin-4, regulators of the immune system, in tobacco cell suspension cultures. According to Magnuson et al (1996):

> "The ability to increase the efficiency of mammalian protein production in plant suspension culture systems should provide significant advantage over protein production in intact transgenic plants which require cultivation, harvesting, and expensive extraction procedures to obtain non-secreted foreign proteins."

Some advantages of plant cell culture over open-air biopharming include (adapted from DeFuría 1996):

1) Faster growth than whole plants
2) Culture conditions easily controlled versus uncontrolled environmental factors
3) Extraction and purification easier than with whole plants
4) Controlled, reliable supply of high quality material
5) Greatly reduced contamination risks versus open-air biopharming

Rhizosecretion

Another contained method for production of biopharmaceuticals and biochemicals is engineering plants to secrete these substances from their roots into hydroponic media, a process known as rhizosecretion. Gleba et al (1999) have experimentally engineered tobacco to rhizosecrete three recombinant proteins – human placental secreted alkaline phosphatase, xylanase and green fluorescent protein (p. 5976). Like plant cell cultures, rhizosecretion is a contained use that poses much less risk of contamination than open-air biopharming. Extraction and purification are much easier and less expensive from hydroponic media than from complex plant tissues. Finally, rhizosecretion is a continuous, non-destructive process offering the prospect of a continual supply of biopharmaceuticals, in contrast to the destructive batch processing necessitated by pharming whole plants (Ibid).

Conclusion

The open-air cultivation of biopharm plants will likely result in contamination of food crops with drugs and chemicals (Section 5.4), posing risks to human health, the environment, and the economic interests of farmers. If there were no alternative, these risks might be deemed acceptable when weighed against the potential benefits of a valuable drug. As we have seen, however, there are a number of ***contained*** production systems, which minimize the risk of contamination: bacterial, yeast, plant and animal cell cultures, as well as rhizosecretion. These systems also permit precise ***control*** of production conditions, which is impossible when growing biopharm plants out of doors,

making it easier to produce biopharmaceuticals of consistent quality. A third advantage is ***ease of extraction***, meaning a cleaner product purified at less cost and effort than is possible from whole plants. Finally, cell cultures and plant root secretions can be ***harvested continuously***, versus the destructive batch processing made necessary by whole-plant biopharming.

A widely touted advantage of open-air biopharming is low production costs. Thus, some will say that market forces favor this production method over more expensive contained systems. One flaw in this argument is that the presumed low cost of open-air biopharming is based on externalization of the costs and liability risks of contamination, which as we have seen is likely to occur in many cases. This externalization, in turn, has thus far been facilitated by the failure to enforce meaningful gene containment regulations, and the failure to make biotechnology companies liable for genetic contamination. If the costs imposed on farmers by the spread of biotech and biopharm traits to non-GMO and organic crops were internalized, market forces would probably no longer favor this uncontrolled production system. A second market-distorting force is the heavy subsidization of biopharm companies in the U.S. (Section 7.2.4), despite their failure to produce any marketable products beyond two esoteric research chemicals with extremely small markets. If the contained methods described above enjoyed similar financial support from government, they might very well end up being the production systems "selected by market forces." Government agencies such as the USDA, FDA and NIH that subsidize research and development in this area have a duty to consider the potential health and environmental impacts of the various production methods and incorporate these criteria in their funding decisions.

"BIOPHARM
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