12
The Liabilities from Regulating Gene Flow in Plant-made Pharmaceuticals

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Introduction

The rate of adoption for genetically modified (GM) crops at the close of the 20th century is arguably one of the most defining moments in the history of agriculture within that century. This rapid global adoption of GM varieties of canola, corn, cotton, and soybeans was not without controversy though. At the opening of the 21st century, the debate regarding contentious issues shows no signs of abating. The number of contentious issues is wide and varied, ranging from social issues, such as consumer acceptance, to scientific issues like gene flow.

Debate about gene flow has grown in importance over the past several years, especially with the commencement of crop trials involving pharmaceutical plants. There are two justifications for pursuing the technology of pharmaceutical plants. Firstly, production of high-quality biological material is presently carried out using mammalian cells inside a bioreactor, which is very expensive and results in high drug costs that could potentially limit the number of people that benefit from new drugs. Secondly, even at the present time, there is an insufficient level of bioreactor capacity available to meet the current demand, let alone the expected increase in demand over the next decade.

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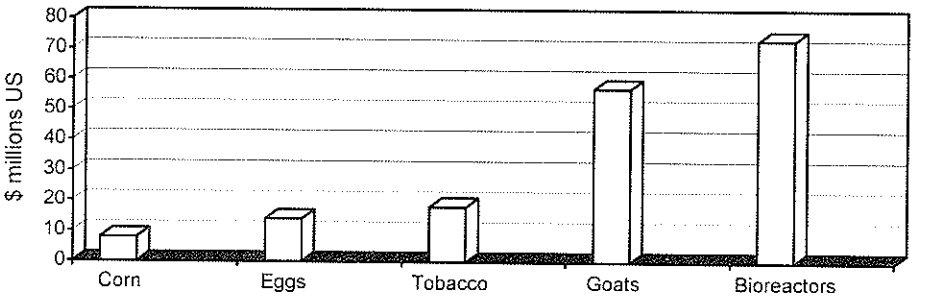
Abbreviations: APHIS, Animal and Plant Health Inspection Services; BIO, Biotechnology Industry Organization; CBI, Council for Biotechnology Information; CT, cholera toxin; DNA, deoxyribonucleic acid; EPA, Environmental Protection Agency; FDA, Food and Drug Administration; GM, genetically modified; HbsAg, hepatitis B surface antigen; IFN, interferon; LT, labile enterotoxin; NAFTA, North American Free Trade Agreement; NIH, National Institutes of Health; PMPs, plant-made pharmaceuticals; PNT, plant with novel trait; RSV, respiratory syncytial virus-F; TGEV, transmissible gastroenteritis coronavirus.

Biotechnology and Genetic Engineering Reviews – Vol. 21, November 2004
0264–8725/04/21/277–297 $20.00 + $0.00



Source: Pew Initiative on Food and Biotechnology, 2002.

Figure 12.1. Cost of various antibody production systems (US$M to produce 300 kg).

Figure 12.1 compares the costs of the various antibody production systems, which are valuable for the treatment of arthritis, herpes, and cancer. The prevailing technology of using mammalian cells to produce human antibodies costs in the range of US$105–175 per gram. It has been estimated (McCloskey, 2002) that transgenic plants might be able to produce the same amount of antibodies at a cost of US$15–190 per gram. The range of variation in anticipated plant-made pharmaceutical (PMP) costs arises from the prospect that the use of PMPs will lower production costs to a level that is economically feasible for potential new proteins that are presently prohibitively expensive to produce. Mammalian cell bioreactors take an average of five to seven years to build at a cost of US$600 million, and McCloskey estimates that, at present, 20 to 50 products could be delayed due to the unavailability of bioreactor capacity. The lack of production capacity is confirmed by the fact that production of four pharmaceutical products requiring biologics presently consume 75% of mammalian cell fermentation capacity. By the end of this decade, there could be more than 80 competing antibody-dependent products, with an estimated value that could exceed US$20 billion. The size of the market underlies the importance of exploring the potential of developing pharmaceutical plants.

As the economic importance of producing human antibodies grows, and there are an increasing number of pharmaceutical plants grown commercially, the regulation of the plants will become a crucial issue. Of paramount importance will be assurances that the production of pharmaceutical plants will not co-mingle with conventional crop production destined for human consumption. The detection of drug proteins in processed food products could destroy the social trust in pharmaceutical crop technology, and ultimately destroy the ability to take advantage of this technology.

The importance of innovative human therapeutics

Proteins of human therapeutic value, interferon and insulin, were the first products of recombinant DNA technology to be produced in bacteria. The justification, at the time (mid 1970s), was that the fermentation and downstream processing technologies were mature, convenient, and cost effective. The techniques of biotechnology were later used in animals, in 1980, and in plants, in 1983. These techniques of biotechnology had multiple applications, many of which, at least initially, had

Table 12.1. Comparison of production systems for recombinant human pharmaceutical proteins

System	Overall cost	Production timescale	Scale-up capacity	Product quality	Glycosylation	Contamination risks	Storage cost
Bacteria	Low	Short	High	Low	None	Endotoxins	Moderate
Yeast	Medium	Medium	High	Medium	Incorrect	Low risk	Moderate
Mammalian cell culture	High	Long	Very low	Very high	Correct	Viruses, prions, and oncogenic DNA	Expensive
Transgenic animals	High	Very long	Low	Very high	Correct	Viruses, prions, and oncogenic DNA	Expensive
Plant cell cultures	Medium	Medium	Medium	High	Minor differences	Low risk	Moderate
Transgenic plants	Very low	Long	Very high	High	Minor differences	Low risk	Inexpensive

Source: Ma *et al.*, 2003.

little direct relationship to clinical medicine. Although initially promising, the production of human therapeutic proteins such as antibodies in mammalian cells or animals (cows, goats, pigs, and sheep) has not, as yet, had a wide adoption. Antibodies produced in mammalian expression systems are expensive and difficult to scale up, and pose safety concerns due to potential contamination with pathogenic organisms or oncogenic DNA sequences. Plants, on the other hand, have become the principal focus for the production of antibodies, enzymes, vaccines, and other therapeutic agents. With advances in transformation and expression systems, it can be expected that more pharmaceutically important genes from many species will be inserted into plants. *Table 12.1* provides a comparison of the existing recombinant human protein production systems.

It is estimated that there are over 250 000 higher plant species, which are represented by a diversity of 300 different families. Natural products of botanical origin have included antimalarial drugs, aspirin, digitalis, and the anticancer compound vinblastin, to name just a few. Overall, 25% of the prescription drugs in the US$300B pharmaceutical industry have plant origins (Cap *et al.*, 2002). Plants represent a cost-effective, convenient, and safe alternative production system for several therapeutic vaccines, enzymes, and recombinant antibodies (plantibodies). According to industry reports, these are the fastest growing class of new medicines for the treatment and prevention of human diseases, such as Crohn's disease, rheumatoid arthritis, cancer, and other potential ailments (e.g. inflammatory, central nervous system, cardiovascular, and infectious diseases). *Table 12.2* shows examples of more than 40 recombinant proteins currently used in the treatment of human diseases, or that are presently in the first or second phase of clinical trials.

There are ample demonstrations of plants and major food crops being efficiently transformed to produce, accumulate, and store fully assembled and functional candidate transgenic products (Khachatourians *et al.*, 2002). Transgenic plants have become the alternate system for increasing the production capacity and large-scale production and processing systems for antibodies (Faye *et al.*, 2003). The expression of immunotherapeutic proteins in plants has two major advantages compared to other expression systems: first, there is no risk of contamination with mammalian viruses or other pathogens; and second, the production of high amounts of antigens is cheap and, therefore, of great economic interest.

Table 12.2. Plant-based protein pharmaceuticals

Proteins	Host plant	Clinical use	Reference
Anti-RhD IgG1 antibody	–	Alloimmunization RhD– mothers with RhD+ fetus	Bouquin *et al.*, 2002
Cholera toxin (CT)	–	–	Chikwamba *et al.*, 2002
Heat labile enterotoxin (LT)	maize	–	–
Hepatitis B surface antigen	potato	Hepatitis	Richter *et al.*, 2000
HuIFN-alpha	potato	*L. monocytogenes* infection	Ohya *et al.*, 2003
Measles virus haemagglutinin	carrot	Measles	Marquet *et al.*, 2003
Respiratory syncytial virus-F	tomato	Respiratory infections, infancy/early childhood	Sandhu *et al.*, 2000
Spike protein (N-gS)	potato	Swine TGEV	Gomez *et al.*, 2000

Although edible vaccines seem to be feasible, antigens of human pathogens have mostly been expressed in plants that are not attractive for human consumption (such as potatoes) unless they are cooked. Boiling may reduce the immunogenicity of many antigens. More recently, the technologies to transform fruit and vegetable plants have been perfected (Khachatourians *et al.*, 2002) to make plant products that can be eaten raw, paving the way towards edible vaccines. Delivery of immunotherapeutic pharmaceuticals in edible plant seeds is a legitimate source for oral vaccines, because antigens have been shown to be correctly processed in plants into forms that elicit immune responses when fed to animals or humans. Antigens expressed in corn are particularly attractive since they can be deposited in the corn seed, and can be conveniently delivered to any organism that consumes grain. Edible vaccines can be used for active and passive immunization. *Table 12.3* highlights some of the American-based firms that are presently conducting research using plant-made pharmaceuticals.

The cost of production of a new antibody requiring new manufacturing facilities is close to US$100M, and can take up to 4 years before being capable of production. The comparative cost of production of one kilogram of an antibody through a conventional bioreactor would require an estimated US$3.75M, whereas the same product generated in corn would cost US$33 000 (Hileman, 2002). Large-scale production of pharmaceutical-grade antibodies requires further focus on development and operation of robust, reliable, and cost effective processes and their validation (Fahrner *et al.*, 2001). Plant produced therapeutic antibodies are already in clinical trials, but account for a very small percentage of the overall production from genetically engineered crops. Current estimates of transgenic food crops are nearly 60 million ha, based on production in 2002.

Plant-made pharmaceuticals are moving into the mainstream of biopharmaceutical manufacturing technologies. Daniell *et al.* (2001) review medical molecular farming, production of antibodies, biopharmaceuticals, and edible vaccines in plants, arguing that they are cheap to produce and store, easy to scale up for mass production, and safer than those derived from animals. Here, we discuss recent developments in this field and possible environmental concerns.

Table 12.3. Companies producing plant-based protein pharmaceuticals

Company	Location	Proteins	Host plant
AltaGen Bioscience	Mirgan Hill, CA, USA	Antibodies	–
Dow AgroSciences Co.	Midland, MI, USA	Proteins, antibodies	–
EPicyte Pharmaceuticals	San Diego, CA, USA	Antibodies	Corn
Exelixis Plant Sciences Inc.	San Francisco, CA, USA	Various proteins	*Arabidopsis*
Integrated Protein Technologies	St. Louis, MO, USA	Antibodies	Corn
Meristem Therapeutics	Clermont-Ferrand, France	Enzymes	–
Monsanto	St. Louise, MO, USA	Antibodies, hormones, proteins	Tobacco, corn
PhytaGenics	Richland, WA, USA	Plasma proteins, wound sealant	–
ProdiGene	College Station, TX, USA	Enzymes, vaccine	Corn
Scale Biology Corporation	Vacaville, CA, USA	Enzymes	–
Sunol Molecular Corp.	Miramar, FL, USA	Antibodies, anti-tissue factor	–

Plants with engineered pharmaceutical secondary metabolite products

Studies of plant secondary metabolites have been increasing over the past 50 years. These molecules are known to play a major role in the adaptation of plants to their environment, but also represent an important source of active pharmaceuticals. Plant cell culture technologies were introduced at the end of the 1960s as a possible tool for both studying and producing plant secondary metabolites. Despite all of the efforts of the past 30 years, plant biotechnologies have led to very few commercial successes for the production of valuable secondary compounds. Different strategies, using *in vitro* systems, have been studied extensively with the objective of improving the production of secondary plant compounds. Biotechnology has opened a new field, metabolomics, with the possibility of directly modifying the expression of genes related to biosynthetic pathways that lead to secondary plant metabolites and metabolic engineering. The delivery of PMPs could occur through ingestion of raw plant material, or from a full, or possibly partial, conventional processing method.

Plants with engineered immunotherapeutic products

Unlike the past, where undifferentiated cell cultures were the target for the engineering of transgenic products, current interest and technologies have provided the opportunity to exercise the option for PMP production in leaves, tubers, fruits, hairy roots, and other organs.

LEAVES

Yusibov *et al.* (2002) showed immunogenicity of plant virus-based experimental rabies vaccine obtained in *Nicotiana benthamiana* and spinach (*Spinacia oleracea*) plants and used for parenteral immunization of mice. Mice immunized with recombinant virus were protected against challenge infection. Based on the previously demonstrated efficacy of this plant virus-based experimental rabies vaccine, when orally administered in virus-infected unprocessed raw spinach leaves to mice, significant antibody responses were demonstrated to the rabies virus. These findings provide a clear indication of the potential of the plant virus-based expression systems as a supplementary oral booster for rabies vaccinations.

TUBERS

Microorganisms such as viruses, bacteria, and certain protozoa cause diarrhoeic diseases of humans and animals. Gomez *et al.* (2000) have shown oral immunogenicity of the spike protein from swine-transmissible gastroenteritis coronavirus (TGEV) in potato tubers. Mice inoculated intraperitoneally, or fed directly, developed serum antibodies specific for TGEV-gS protein, demonstrating the oral immunogenicity of the plant-derived product. A different strategy for multivalent vaccine production from food plants for simultaneous protection against infectious virus and bacterial diseases was engineered by Yu and Langridge (2001). They created a fusion of cholera toxin (CT) B and A2 subunit with a rotavirus enterotoxin and enterotoxigenic *Escherichia coli* fimbrial antigen genes engineered into potato plants. Orally immunized mice indicated the presence of a strong immune response to the three

plant-synthesized antigens. Diarrhoea symptoms were reduced in severity and duration in passively immunized mouse neonates following rotavirus challenge.

There are several programmes attempting to achieve global immunization for hepatitis B prevention and eradication. Oral immunization for hepatitis B virus with an 'edible vaccine' is an important strategy in this area. Richter *et al.* (2000) showed oral immunogenicity of recombinant hepatitis B surface antigen (HBsAg) in preclinical animal trials. Mice fed transgenic HBsAg potato tubers showed a primary immune response (increases in HBsAg-specific serum antibody) that could be boosted greatly by intraperitoneal delivery of a single sub-immunogenic dose of commercial HBsAg vaccine, indicating that plants expressing HBsAg in edible tissues may be a new means for oral hepatitis B immunization. Kong *et al.* (2001) performed oral immunization with hepatitis B surface antigen (HBsAg) expressed in transgenic potato plants. In fact, in transgenic plants HBsAg accumulated intracellularly, suggesting natural bioencapsulation of the antigen as a means to provide protection from degradation in the digestive tract. These authors show transgenic plant HBsAg material to be superior in both inducing a primary immune response and priming the mice to respond to a subsequent parenteral injection of HBsAg. Marquet *et al.* (2003) use the desirability and palatability of edible vaccines containing antigens of human pathogens when they are expressed in crops that must be cooked. Boiling may reduce the immunogenicity of many antigens. They suggest that transformed carrot plants are a better choice. They were successful in the production of the immunodominant antigen of the measles virus. Their study may pave the way towards an edible vaccine against measles, which could be complementary to the current live-attenuated vaccine.

SEEDS

Chikwamba *et al.* (2002) showed the ability of transgenic maize plants to produce enteropathogenic and diarrhoea-causing *E. coli* heat labile enterotoxin (LT) and CT for use as an edible vaccine. Orally immunized mice had mucosal and systemic immune responses, and survived challenge by oral administration of the diarrhoea-inducing toxins.

FRUITS

Hepatitis B is a serious and debilitating disease, which can be prevented through vaccination. However, the cost and feasibility of widespread vaccination are problematic. Gao *et al.* (2003) provide some theoretical and experimental directions for the production of large-scale, low-cost oral hepatitis B (HBsAg) vaccine using transgenic cherry tomatillo. Sojikul *et al.* (2003) engineered a signal peptide from soybean, a vegetative storage protein-HBsAg fusion protein, with enhanced stability and immunogenicity expressed in tobacco plant cells.

Sandhu *et al.* (2000) found oral immunization of mice with transgenic tomato fruit expressing respiratory syncytial virus-F (RSV) protein induces a systemic immune response. Here, a fruit-based, edible subunit vaccine against RSV was developed by expressing the RSV fusion (F) protein gene in transgenic tomato plants. The F-gene was expressed in ripening tomato fruit under the control of the fruit-specific E8

promoter. Oral immunization of mice with ripe transgenic tomato fruits led to the induction of both serum and mucosal RSV-F specific antibodies.

Plants with engineered human pharmaceutical enzyme products

LEAVES

A number of inborn errors in metabolism and enzyme deficiencies in humans can be corrected by the provision of enzymes. An example is Fabry's disease, a human lysosomal storage disorder. Human alpha galactosidase is used to treat individuals with Fabry's disease. Unlike beta galactosidase, which is found in many bacteria and fungi, alpha galactosidase is scarce, and its production very expensive. Tobacco plants engineered to produce this enzyme can yield 50 mg of enzyme per kg of freshly harvested tobacco plants (Potera, 2003). The US Food and Drug Administration (FDA) has granted Large Scale Biology Corp. of Vacaville, California, USA orphan drugs status for the production of this therapeutic enzyme.

SEEDS

ProdiGene Corporation is already in commercial scale-up of two proteins, the enzyme trypsin and aprotinin, an inhibitor of protease, from GM corn. Trypsin is used in the production of insulin, as an industrial protease, and is also used as an oral medicine for wound care. Trypsin is usually produced from bovine pancreas. Aprotinin, used in cardiac surgery to prevent blood loss, and also used in wound healing, is obtained from bovine lungs.

PMP-peptides

Bioactive peptides are 3 to 40 amino acids in length, and carry a range of biological properties commercially useful to life and clinical sciences, and agri-food industries. Areas of research and development of current interest are drugs active as hormones, insulin, anticoagulants, vasopressins, brain neuropeptides, and anti-microbial peptides. Peptide science has matured and, in the years to come, should have significant value to the pharmaceutical industry (Rocchi and Gobbo, 2002). The major method for large-scale manufacturing of peptides is still by chemical synthesis, or by fermentation of recombinant microorganisms (Verlander, 2002).

TUBERS

Material costs and scale-up of plant-based peptide production argues favourably for the use of plant systems and transgenics. Ohya *et al.* (2003) have shown type I interferon (alpha/beta IFN) production in transgenic potato. This is the first cytokine used for clinical applications against autoimmune, viral, and neoplastic diseases through oral administration.

LEAVES

deGray *et al.* (2001) showed expression of an antimicrobial peptide via the

Table 12.4. History of pharmaceutical field trials

Country	History of pharmaceutical crops trials											
	Total	2002	2001	2000	1999	1998	1997	1996	1995	1994	1993	1992
USA	62	7	18	14	12	8	1	1	–	–	–	1
Canada	53	6	3	6	8	11	5	11	2	1	–	–
Japan	7	–	–	7	–	–	–	–	–	–	–	–
France	6	–	1	–	3	–	1	–	1	–	–	–
Argentina	3	–	–	–	–	3	–	–	–	–	–	–
Australia	1	1	–	–	–	–	–	–	–	–	–	–
Italy	1	–	1	–	–	–	–	–	–	–	–	–
Spain	1	–	–	–	–	–	1	–	–	–	–	–
Total	134	14	23	27	23	22	8	12	3	1	0	1

Source: APHIS, 2003; CFIA, 2003; CONABIA, 2003; European Commission, 2003; Japanese MAFF, 2003; OECD, 2003; OGTR, 2003.

chloroplast genome to control phytopathogenic bacteria and fungi. Antimicrobial peptide MSI-99, an analogue of magainin 2, was expressed via the chloroplast genome to obtain high levels of expression in transgenic tobacco plants. Genetically engineering crop plants for disease resistance via the chloroplast genome instead of the nuclear genome is desirable to achieve high levels of expression, and to prevent pollen-mediated escape of transgenes.

Global status of field trials involving crop plants producing pharmaceuticals

Pharmaceutical field trials began in 1992 in the United States (*Table 12.4*). In North America, the use of pharmaceutical trials was limited through the early to mid 1990s. Use of these trials took off in Canada beginning in 1996, but declined following 2000. Correspondingly, in the US, trials became more common in 1998, and appear to have declined following 2001. The period from 1995–2002 has witnessed sporadic use of pharmaceutical field trials by a host of other countries.

There are several reasons for variations in the number of field trials during the past decade. First, the issuance of approvals differed amongst nations and regions. Second, the pace of discovery can be serendipitous or planned. The crop pharmaceutical industry itself is in the early stages of development, so there is great uncertainty and lumpiness in the need for trials. As such, field trials, as with conventional pharmaceutical clinical trials, are unlikely to have a predictable trend. Third, variations in field trials can occur because seasonal and/or environmental conditions can dictate postponement of trials. Fourth, as judged by the analysis of the crop kinds, we should expect fluctuations in the numbers of pharmaceuticals that could be derived from any one crop. The genetic and physiological constraints in plants place limits on their use for transgenic plant construction, both in food and pharmaceutical contexts (Khachatourians *et al.*, 2002).

There was no identifiable advantage for either Canada or the US in the early stages of PMP crop research (*Figure 12.2*). Canada enjoyed a research advantage from 1996–1998, while the US enjoyed the advantage from 1999–2001. Both countries converged again in 2002. Canada's early advantage was derived from the research conducted with canola, which proved in the late 1990s to present too many safety

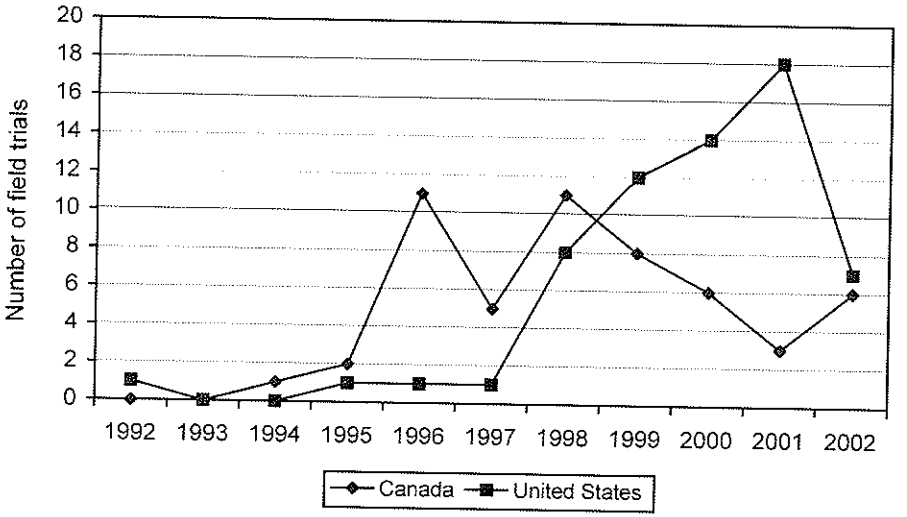


Figure 12.2. Competitive advantage comparison in pharmaceutical trials.

concerns to proceed. The US lead was based upon research using corn as the plant of choice between 1999 and 2001. By 2002, corn appeared to be falling out of favour in the US for crop trials, while trials are climbing in Canada again based on field trials with safflower.

The evolution of PMPs has mirrored the research trends in agricultural biotechnology: transformation research started with tobacco; moved to dicotyledons such as canola; and finally to monocotyledons, where the first research was with rice. The different crop varieties used for pharmaceutical trials are shown in *Table 12.5*. Canola was the early favourite, due to the amount of canola transformation research that had already taken place, and its attractive oil properties. After several years of pharmaceutical crop experimentation in Canada, it became obvious that canola was not a suitable host plant due to the high incidence of pollen flow, and the threat posed to the large production of canola in western Canada. At the time that pharmaceutical canola trials were ending, trials started with corn, rice, and tobacco. The use of corn and tobacco for pharmaceutical trials grew between 1997 and 2001, while experiments in rice have been minimal. Experiments with the use of flax occurred briefly in Canada in the late 1990s, but concerns about pollen flow once again removed the potential of further trials. Trials in safflower have commenced in the past 3 years as this research has replaced the previous canola research. A variety of other crops, such as forages, vegetables, and flowers, have been experimented with, but it would seem that little in the way of useful pharmaceutical potential is available in these plant varieties. The one exception to this may be the use of poppies in Australia for improved production of opium. Field trials of transgenic pharmaceutical poppies started in 2002, and it would appear that there is some long-term potential with the use of poppies. *Figure 12.3* provides a summary of global pharmaceutical variety trials by crop.

There was a noticeable decline in pharmaceutical crop trials in 2002. There may be three reasons for this. First, crop trials are cyclical by their very nature, and this

Table 12.5. Historical perspective of pharmaceutical crop kinds

Crop	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Alfalfa	1	–	–	–	–	–	3	–	–	–	–
Barley	–	–	–	–	–	–	–	–	–	1	–
Canola	–	–	1	2	2	8	4	–	–	–	–
Clover	–	–	–	–	–	–	–	–	–	1	–
Corn	–	–	–	–	–	1	5	11	11	14	4
Flax		–	–	–	–	–	1	1	2	–	–
Mustard	–	–	–	–	1	–	2	–	–	–	–
Poppy	–	–	–	–	–	–	–	–	–	–	1
Rice	–	–	–	–	–	1	2	2	2	2	–
Safflower	–	–	–	–	–	–	–	–	1	2	4
Sugar cane	–	–	–	–	–	–	–	–	–	1	–
Tobacco	–	–	–	1	–	1	–	5	5	9	4
Tomato	–	–	–	–	–	–	1	–	–	–	–

Source: APHIS, 2003; CFIA, 2003; CONABIA, 2003; European Commission, 2003; Japanese MAFF, 2003; OECD, 2003; OGTR, 2003.

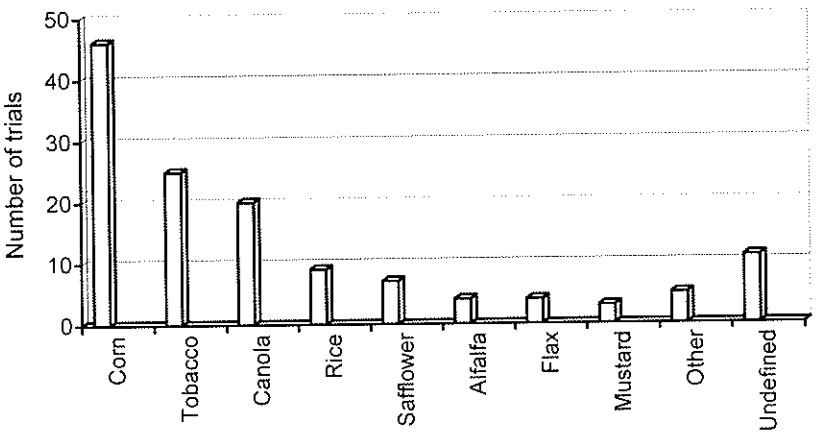


Figure 12.3. Breakdown of pharmaceutical trials into crop kinds.

may be nothing more than a natural dip in the number of trials. Second, drug companies have been conducting trials for 5–6 years with some crop kinds, have now completed Phase 3 clinical trials, and are waiting to see what the financial outcome will be prior to commencing new research. Third, drug companies want to control the pipeline for this technology. By limiting the number of trials, the demand for the products grows, and thus increases the size of the potential market.

Challenges of pharmaceutical crop production

The initial contentious issue regarding gene flow was canola (Smyth *et al.*, 2002), and that situation remains unchanged. Scientists and regulators are still in a conundrum at best, or conflict at worst, about the impacts and regulations of gene flow. The three leading crop kinds used for pharmaceutical trials have been corn, tobacco, and canola. The problem with these is that corn and canola are crop kinds intended for human consumption, and the potential for co-mingling or cross-

Table 12.6. Transgenic crops and human consumption

Transgenic crop category	Specific transgenic crops	Use in pharmaceutical trials	Cross-pollination potential	Modality of consumption	
				Plant tissue(s) and organs*	Extracellular plant metabolic ingredients
Cereals	Corn, barley, rice, wheat	Yes	Low–medium	Direct	No
Oilseeds	Canola, flax, mustard, cotton, safflower	Yes	High	Mainly indirect	Yes
Pulses	Soybean	Yes	Low	Direct and indirect	No
Forages	Alfalfa, clover, tobacco, sugar cane, sugar beets	Yes	Medium	Very minimal indirect	No
Fruits and vegetables (including juices)	Poppy, cantaloupe melon, radish, potato, squash tomato, strawberry, lettuce and papaya	Yes	Low–high	Direct	Yes

*Note: Meaning plant cells, tissues, and organs, including rDNA or primary or secondary metabolites (i.e. oils, starches, proteins, amino acids, and processed materials and tissues, including juice).

pollination exists, raising concerns about the use of these crop kinds for pharmaceutical trials. *Table 12.6* presents the major transgenic crop kinds, identifies whether they are used in pharmaceutical trials, and examines the modality of consumption.

The leading transgenic cereal crop is corn, where a total of 9% of global corn acres are transgenic and grown in seven countries (James, 2003). The leading transgenic oilseed is canola, which is grown in Canada and the US; transgenic canola accounts for 12% of global canola acres. Soybean, the sole transgenic pulse crop, is grown in seven countries and accounts for 51% of the global soybeans acres. The clear leading transgenic forage crop is tobacco, while a variety of fruits and vegetables are beginning commercial production.

Transgenic cereals, fruits, and vegetables are, for the most part, consumed directly. The majority of cereals are consumed directly, such as corn and rice. Transgenic fruits and vegetables are also consumed directly, while some processing would occur in the juice-making process. Transgenic oilseeds are largely used to produce processed oils, which are used to fry food. Most pulses are consumed directly, but the only transgenic pulse, soybean, is used largely for animal feed and in the production of tofu. Forage products are rarely consumed by humans, with a small amount of alfalfa sprouts being the exception.

As the technology of pharmaceutical and transgenic plants rapidly moves from laboratory to field, the regulations developed to control these new crop varieties were severely tested in 2002. While regulators in the US have argued that the detection of ProdiGene's experimental pharmaceutical corn in a silo of soybeans late in 2002 is proof that the regulations are working, the simple fact that a pharmaceutical crop that was supposed to be contained on-farm actually reached a grain terminal without being detected shows that the regulations are not stringent enough.

Table 12.7. Impact of transgenic crop regulatory violations

Company	Crop	Location	Violation	Impact
ProdiGene	Corn	Nebraska	Volunteer corn growing in soybean field	Fined US$250 000 and forced to pay clean-up costs of US$3.5 million
Dow AgroSciences	Corn	Hawaii	No tree windbreak and bordering rows	Fined US$8800
Pioneer Hi-Bred	Corn	Hawaii	Plot planted in unapproved location	Fined US$9900
Dow AgroSciences	Corn	Hawaii	Plants detected with unapproved gene and failure to promptly notify the EPA	Fined US$72 000

The containment of living plants is proving to be increasingly challenging, given humans' inability to completely control nature.

The issue of unintended gene flow became a global news issue in the fall of 2001 with the discovery that some varieties of Mexican maize contained transgenic material that should not have been there (Quist and Chapela, 2001). [Note: This research is contested within the scientific community, and is presently the subject of a NAFTA review.] This topic continued to be important into the summer of 2002 as a research team led by Allison Snow of Ohio State University reported preliminary evidence suggesting that the trait from transgene insertions in sunflowers may be able to move to other plants, thus creating the conditions for 'superweeds' (Snow *et al.*, 2003).

The problem multiplied in the fall of 2002 and spring 2003 (*Table 12.7*). In November 2002, ProdiGene Inc. was fined US$250 000 for allowing experimental pharmaceutical corn volunteers to grow to maturity within a soybean field. Inspectors with the United States Department of Agriculture's Animal and Plant Health Inspection Servces (APHIS) discovered the regulatory infringement. The resulting soybean crop was harvested and pooled in a commercial grain silo, thus contaminating an estimated 500 000 bushels of soybeans. The cost to ProdiGene for buying the contaminated soybeans and having them transported to be destroyed was an estimated US$3.5 million.

Within a month, the topic was once again making news headlines in North America. In mid December, the US Environmental Protection Agency (EPA) fined Dow AgroSciences and Pioneer Hi-Bred for two separate regulatory violations. Dow was fined US$8800 for failing to meet all the defined conditions to prevent gene transfer with an experimental transgenic corn variety undergoing field trials at Molokai, Hawaii. The plot, which was 1/10th of an acre in size, failed to meet the EPA permit conditions because there was no windbreak made of wiliwili trees in place, and the bordering rows (the outside 12–24 rows) were not of the variety specified in the permit. Pioneer was fined US$9900 for an experimental transgenic corn variety in Kauai that was planted in an unapproved location that turned out to be too close to other experimental corn varieties. The Pioneer permit from the EPA specified an isolation distance of 1260 feet, and this distance was not observed.

Finally, in April 2003, Dow was again fined for violating the EPA permit in Kauai. This time the fine was US$72 000, and resulted from the detection of 12 transgenic

corn plants that contained an unapproved gene that is suspected of coming from the pollen from another experimental plot located nearby. Although Dow officials discovered this unplanned gene flow, Dow failed to notify the EPA promptly, and EPA officials expressed disappointment over the delayed response by Dow. When this incident was reported in the *Washington Post* (24 April, 2003), the article stated that this incident was '... the latest setback for a biotechnology industry struggling to comply with government rules. ... some advocates say the problems cast doubt on a fundamental premise of government policy: that experimental varieties of corn or other crops can be planted in fields but kept out of food crops.'

Four separate, but related, regulatory violations within a six-month period may be nothing more than a freak statistical occurrence that may never be observed again. What is more troubling, and likely more representative of the real issue, is that these regulatory violations could simply be the tip of the iceberg, and that evidence of these regulatory violations could continually be documented for the foreseeable future.

Defining liability

Legally, a liability results when an obligation is not fulfilled. From this legal perspective, there are only two kinds of liability, criminal and civil. Criminal liability is when there has been a criminal act committed because the guilty party broke the obligation of the law of the land. Civil liability is when a party has not met an obligation, and this can result in litigation seeking compensation on behalf of those affected. Lawsuits from those affected by thalidomide and silicone breast implants are examples of civil liability.

The issue of strict liability can also be relevant to regulatory violations. Typically, strict liabilities are found for one-time occurrences, such as is the case of *Rylands* v. *Fletcher* (1868), LR 3 HL 330, affg LR 1 Ex. 265. In this case from Britain, Fletcher owned a mill and to supply it with water, he constructed a reservoir. During the construction of the reservoir, the contractors discovered five long abandoned vertical shafts, not knowing they were abandoned mine shafts, the shafts were filled with soil. The reservoir was partially filled and, shortly after, one of the soil filled shafts gave way, flooding the nearby coal mine owned by Rylands. Rylands sued Fletcher for the destruction of his mine. The ruling in *Rylands* v. *Fletcher* describes the item or product being stored on one's land as not naturally occurring, and therefore, this product is inherently dangerous. In this case, a large inland body of salt water was viewed as a dangerous activity.

This ruling has three important considerations for the gene flow of pharmaceutical crops. First, the drift of transgenic pollen is not a one-time occurrence; rather, it happens annually, for a period of 3–6 weeks in most crop varieties. Secondly, it would seem impossible to argue that transgenic pollen is stored in any form or fashion upon a farm or a field test plot. Third, the presence of pharmaceutical genes in crops destined for human consumption could be inherently dangerous. The key argument from *Rylands* v. *Fletcher* was that the danger was not naturally occurring. There is a strong argument to be made that pharmaceutical crops are not naturally occurring and that the unintended gene flow from these crops may be inherently dangerous. There would be a duty of care owed by the producer of the PMP crop, and a liability suit could be brought on the basis of nuisance and/or negligence.

However, while many will say that putting any other adjective in front of liability is meaningless, it can be argued that innovations in transgenics can potentially foster the establishment of socio-economic liability. A socio-economic liability is defined as the decline in social trust for all innovations, and the economic decline from commercialization delays when a company or government regulatory body fails to meet their publicly stated objectives. When a regulatory failure occurs, the resulting media coverage can change the social perception of the innovation. Negative media coverage will result in some consumers that initially were indifferent to a specific innovation becoming concerned about it, or even opposed to the innovation, and possibly to innovation in general. The bottom line is there is a loss of social trust in innovation, albeit specific or general. This is not to say that the decline in trust is long term, or that it cannot be reversed, but rather that there will be fewer consumers willing to express support for an innovation or the resulting commercial products.

Different forms of liability can be classified according to their governance mechanisms. Criminal liability is strictly a legal issue, and dealt with by the courts. In these cases, individuals have broken the law and are either punished financially, or by serving time in a penal institution. Civil liability is an economic issue, and is handled by the court system. However, it is more common for these cases to be settled out of court. Many civil litigation lawsuits are class action suits against large firms, and the court awards financial compensation to those negatively affected, regardless of the company's ability to pay. Executives of the offending firms are not normally sentenced with time in penal institutions in civil litigation suits. Socio-economic liability is not dealt with by the court system, but rather, is reflected by the attitude of the consumers within a given society. Whereas the other two forms of liability are private issues, this aspect of liability is a public issue, as reflected by the loss of trust in a company, a branded food product, or a class of goods.

The absence of criminal or civil liabilities does not negate the fact that when a regulatory failure occurs, there are social externalities that are ultimately borne by other firms, and by consumers. While these externalities may not generate a level of harm that is large or severe enough to trigger litigation for compensation, a socio-economic liability can be created with regulatory failures.

Modelling existing pharmaceutical regulations

The focus of this section will not be on the development of general regulations for biotechnology, other authors have already documented this process. Carpenter and Gianessi (2000) provide one of the most comprehensive reviews for the development of biotechnology regulations that originates with the National Institutes of Health (NIH) in the early to mid 1970s.

The importance of strong governance institutions cannot be understated when the production of a pharmaceutical crop has the possibility of entering the human supply chain. These governance institutions can range from federal regulatory agencies to private industry organizations to judicial systems. An international comparison of the three leading forms of governing institutions (*Table 12.8*) illustrates which institution is the lead institution. The commercialization of transgenic

Table 12.8. Relationship of governance institutions

Country	Type of governance institution		
	Federal regulatory body	Industry association	Judicial system
Canada	Strong	Medium	Strong
United States	Medium	Strong	Strong
France	Strong	Weak	Medium–strong
Australia	Strong	Medium	Strong
Argentina	Weak	Weak	Weak
Japan	Strong	Weak	Strong
Italy	Weak	Weak	Not available
Spain	Medium	Weak	Not available

and pharmaceutical crops varies greatly from country to country, depending upon which institution is the leading institution.

A strong regulatory body is defined as one that anticipates issues of concern to society, and begins to develop regulations prior to the commercialization of products. Strong industry associations are progressive lobby groups that have developed a wide network of industry representation. Strong industry associations will also develop industry standards that may well exceed the regulations provided by government. Strong judicial systems are those that are willing to examine issues relating to the commercialization of transgenic crops and the ownership of the corresponding intellectual property.

The optimum would include three strong institutional pillars that are able to anticipate and manage risks. This would require a strong regulatory body that anticipates issues of concern to society, and begins to develop regulations prior to the commercialization of products. Strong industry associations are also needed to operate as progressive lobby groups with a wide network of industry representation that can develop industry standards that either can become the base for regulations, or can exceed the regulations provided by government. Finally, strong judicial systems are needed to mediate issues relating to the commercialization of transgenic crops, and the ownership of the corresponding intellectual property (in effect, they keep industry operating and accountable).

A closer examination of the regulatory systems in Canada and the US reveals some surprising differences. Some would argue that the Canadian regulatory agencies have been more vigilant regarding transgenic crops than their American counterparts. Beginning in the early 1990s, Canadian regulators stated that all transgenic crops (as well as many mutagenic crops) would be treated as plants with novel traits (PNTs) and, therefore, subject to more regulation than conventional crop varieties. Every new PNT requires mandatory oversight of their trials, efficacy, and impact on safety of food, feed, and the environment. Government agencies demand to see both the raw data and summaries of all tests performed, and have the final say on every introduction. The Canadian system also has a formal system of contract registration for risky industrial crops, and imposes criminal penalties for infractions. While the Canadian regulators have not completed their development of special rules for PMPs, they have been very influential in directing companies away from areas deemed to be of higher risk (e.g. canola) by simply reminding the developers that such products are unlikely to be approved. Meanwhile, the Canadian Council for

Biotechnology Information (CBI) is a smaller association than in the US, and has not developed the synergy that its counterpart, the Biotechnology Information Organization (BIO), enjoys in the US. At least part of the reason is that the concentration of power in the Canadian government (through the Prime Ministerial structure, cabinet secrecy, and party solidarity) limits the association's ability to gain access or find supporters within the government apparatus. While the Canadian judicial system is viewed highly in terms of its independence and professionalism, it is inherently weaker than in the US because of the limited use of class action suits and the very narrow parameters applied for punitive damages.

The initial regulations in the early 1990s in the US were viewed by the industry as being too lax, and therefore insufficient to establish trust with consumers. In response, the industry asked the regulators to strengthen the regulations for transgenic crops. Nevertheless, the American regulatory system has consistently been less rigorous in its approach to dealing with transgenic crops than regulators in Canada – e.g. most reviews are voluntary, non-transgenic novel traits are not reviewed, and the regulatory agencies only see study summaries rather than raw data. As in Canada, the US regulators have not sorted out how to handle PMPs. The extra challenge they face is that they do not have the same powers and legal authority that Canadian regulators have to direct developers away from crops. While the regulatory mechanism may be weaker, the other two domains are stronger. The industry association is considerably larger than in Canada and, given the more open nature of US governance, has better access and a stronger voice in the US than in Canada. BIO is viewed by many as a very authoritative voice when speaking on issues affecting the industry. The courts, similarly, are more engaged, partly because they are more open to class actions, and because they award much higher punitive damages than in Canada. For instance, a class action suit against Aventis in the US claimed that the impacts of StarLink™ had depressed corn prices in the US, and resulted in economic losses for corn producers. Faced with a potentially larger judgment, both parties settled very early into the trial, agreeing on US$110 million in compensation.

On 6 March, 2003, the Animal and Plant Health Inspection Services division of the US Department of Agriculture announced that they would strengthen mandatory permit conditions for field-testing transgenic crops, including field trials for PMPs. The number of site inspections will increase to five during the trial, and two the following season. The permits for pharmaceutical trials will state that no corn can be grown within one mile of the trial site, and that no food or feed crop can be grown on the site the following season. The size of the buffer zone was doubled from 25 to 50 feet. This strengthening of regulatory requirements, in part, can be seen as a method to address the concerns that arose following the regulatory violations between 2001 and 2003.

With the exception of Canada and the US, all other countries have only conducted a very few pharmaceutical crop trials, and this creates a challenge when trying to evaluate the relative strength of the related governance institutions. Three European nations have varying levels of government regulatory bodies. France has been strongly opposed to transgenic crops, and developed strict regulations for transgenic field trials, Italy has changed positions over the past 5 years, and transgenic crops are presently forbidden, while Spain has annually averaged between 45 000 and 55 000 acres of *Bt* corn for the past 5 years (Brookes, 2002). While this is a relatively small

amount of production, it does indicate that the Spanish regulators have developed a functioning regulatory system for the co-existence of transgenic and conventional cropping. The main industry association in Europe, EuropaBio, is a loose coalition of biotechnology firms operating in Europe but, due to the high level of organized opposition, diverse nature of the EU, and widely dispersed power and authorities in the EU, its voice is not heard loudly. The French judicial system has, albeit with a limited number of cases, protected the integrity of research and field trials of transgenic crops (ensuring the isolation of trials, even from protestors, is a foundational requirement for any effective regulatory regime), while the court systems in Italy and Spain have not been tested.

The countries of Australia, Argentina, and Japan have allowed pharmaceutical trials to take place, but on a very limited basis. Australia's regulatory agency is modelled to some degree on those of North America and, therefore, has adopted a consistent policy for transgenic crops. The recent collapse of Argentina's government has resulted in, at best, chaotic regulations. Japan has a very strong regulatory agency whose decisions are consistent with North American decisions, but lag by a period of several years. Australia has a developing industry association, but it is limited as Australia is just in the initial process of granting commercialization to transgenic crops. Argentina and Japan have virtually no effective industry associations. Australia has a judicial system similar to that of North America, but the federal constitution empowers each Australian state individually to approve or ban transgenic crops, which may possibly create a legal jurisdictional battle, with a number of expected lawsuits against the states enacting moratoriums. Again, the disruption of Argentina's economy has reduced the ability of its judicial system to provide consistent decisions. Japan's judicial system has historically been a strong supporter of biotechnology, but there is growing social concern about biotechnology, and this may be reflected in future court decisions.

Based on an analysis of the Canadian and American governance institutions relating to biotechnology, it can be argued that to have a functioning regulatory system there is a requirement to have strong institutions in all three pillars. Australia is developing a functioning regulatory structure, but only after careful observation of events in North America. All the other countries, France, Argentina, Japan, Italy, and Spain, are lacking a strong institution in at least one of the three governance pillars. This lack of institutional leadership results in an imbalance of authority, which may indicate that either the government agencies have too much regulatory power and are unrealistic in their expectations of biotechnology companies, or that there is no structured bureaucracy capable of making and enforcing consistent policy decisions.

Conclusions

The challenge of PMPs is going to be to structure a fully integrated regulatory system that effectively evaluates, manages, and communicates about the risks of a system, and ultimately one that both enforces and is seen to enforce failures. In spite of the US regulatory changes, there is an apparent inability of regulators to enforce the regulations. In the ProdiGene case, the cost of the fine, clean up, and destroying the contaminated soybeans was estimated to be US$3.75 million. The problem with

imposing such a large cost on a small biotechnology company is that there is seldom enough cash flow within the company to pay a fine of this magnitude. The American government had to lend ProdiGene the money to pay the fine. This is symptomatic of the biotechnology industry as a whole, as small biotechnology companies do not have sufficient financial resources to pay large regulatory violation fines. The problem is that, if firms know that governments will provide loans or loan guarantees in the event of fines from regulatory violation, that reduces the incentive for firms to adopt standards that improve the control of pharmaceutical crops. If existing enforcement mechanisms are found wanting or are lacking, trust will be hard to sustain.

Part of the future solutions to the conundrum of PMPs will be stricter regulation. Options such as 1) closed loop system to confine PMPs to select authorized seed processors, certified growers, manufacture sites, and pharmaceutical firms, or 2) production of PMPs in regions where there are no major food crops, are being examined by regulatory systems. The major requirement will be to have three strong governance pillars: government, industry, and courts. An overabundance of power by one of the three governance pillars creates a disequilibrium, and skews the opportunity for balanced regulations.

The challenge would seem to be that in countries where the regulators are unwilling or unable to step forward and be the leading and dominant institution, private industry is shirking the responsibility as proper guardians of new innovations. Similarly, where industry organizations are unable to generate consensus on, or adherence to, proper standards and procedures, governments have often been unwilling or unable to fill these gaps. One option might be to let the legal system step in and establish standards and regulations based on decisions from multiple lawsuits. This may well occur if these stakeholders do not begin to take more seriously their responsibility to society regarding the production of PMPs.

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