Opinion TRENDS in Biotechnology Vol.22 No.2 February 2004

On risk and plant-based biopharmaceuticals

Robert K.D. Peterson[1] and Charles J. Arntzen[2]

[1]Agricultural and Biological Risk Assessment, Department of Entomology, Montana State University, Bozeman, MT 59717-3020, USA
[2]Arizona Biodesign Institute, Arizona State University, Tempe, AZ 85287-1601, USA

Research into plant-based expression of pharmaceutical proteins is proceeding at a blistering pace. Indeed, plants expressing pharmaceutical proteins are currently being grown in field environments throughout the USA. But how are these plants and proteins being assessed for environmental risk and how are they being regulated? Here, we examine the applicability of the risk assessment paradigm for assessing human and ecological risks from field-grown transgenic plants that express pharmaceutical proteins.

Research with transgenic plants continues to offer the promise of large-scale production of safe, pure and highly efficacious therapeutic proteins. These proteins are essential for the production of a wide range of biopharmaceuticals, including monoclonal antibodies (MAbs), enzymes, blood proteins and new types of subunit vaccines for preventing infectious diseases.

Biopharmaceuticals, in particular MAbs, are one of the fastest growing classes of therapeutics, with >200 products in clinical evaluation and many more in preclinical development [1]. These products are being developed to treat life-threatening and chronic diseases including arthritis, cancer, infection, inflammation and cardiovascular diseases.

There are currently 12 therapeutic MAbs approved by the United States Food and Drug Administration (USFDA) and these complex multi-subunit proteins are produced in mammalian cell culture facilities. Recent estimates indicate that seven plant-derived antibodies or antibody derivatives have reached advanced stages of product development [2]. Analysts also predict that >70 therapeutic MAbs could be on the market by 2008, requiring production of >10 metric tons of MAbs annually (L. Dry; www.bio.org/pmp/index.asp). If these estimates are accurate, the result could be an unprecedented demand for new manufacturing capacity for MAbs.

Given a projected shortage in manufacturing capacity, plant-based biopharmaceuticals represent a cost-effective alternative to traditional cell-culture production for meeting growing demands for these therapeutic proteins. Furthermore, because of the current advanced state of the technology, plant biotechnology might provide an efficient, high capacity alternative to traditional cell-culture production.

Corresponding author: Robert K.D. Peterson (bpeterson@montana.edu).

The use of plants to produce proteins with potential human and animal pharmaceutical value has been ongoing for more than 13 years [2,3] and the plant species employed and proteins produced have been diverse. Some of the proteins produced in plants include *Streptococcus mutans* surface protein antigen A, hepatitis B surface antigen, *E. coli* heat-labile enterotoxin, Norwalk virus capsid protein, human aprotinin, human collagen, α-interferon and rabies virus glycoprotein [2,3]. Plant species used for pharmaceutical protein production include tobacco, carrot, tomato, maize, potato, alfalfa, soybean and rice [4].

Much of the research activity associated with plant-based biopharmaceuticals is currently being conducted in laboratory and greenhouse facilities. However, in 2002 20 permits (130 acres on 34 sites) were issued by the United States Department of Agriculture (USDA) for field propagation of plants engineered to produce pharmaceutical proteins [5].

The numerous advantages of using plants to produce pharmaceutical proteins have been extensively reviewed [2–4]. Plants, and crop species in particular, potentially offer extremely cost-effective and efficient production for pharmaceuticals. The genetics, breeding, inherent toxins and anti-nutritients, exogenous contaminants and agronomic production are well understood for many crop species. In addition genetic engineering and expression systems for many crop species are well understood. If pharmaceutical proteins are produced in seeds, the scale-up protein production purification can be conducted at a central site to which harvested material can be shipped. For products to be delivered orally, freeze-drying of plant tissues yields product that does not require cold-chain storage of the final product, and the plant matrix surrounding the protein drug can be considered an excipient or inert carrier. Finally, protein production in plant cells will probably be safer than traditional techniques because of the lack of contamination with extraneous viral or bacterial materials, mammalian pathogens and other animal cell-culture contaminants. These factors mean protein production costs in plants could be reduced by as much as 1000-fold compared with traditional production practices [3].

Plant-based versus traditional production

Production of pharmaceutical proteins in plants represents a paradigm shift for the production of pharmaceuticals and biologics, but also in the uses of products formerly limited to food or animal feed uses. Although depictions in the

 0167-7799/$ - see front matter doi:10.1016/j.tibtech.2003.11.007

mass media might suggest otherwise, the plant (or food) is not the final pharmaceutical product, just as microbial or yeast pharmaceutical production systems do not represent the final product. Rather, the plant represents one step in a complex, multi-step pharmaceutical production process. Consequently, most processes in the production of pharmaceuticals will follow traditional regulatory requirements regardless of whether the proteins are produced using plants or other methods.

…most processes in the production of pharmaceuticals will follow traditional regulatory requirements regardless of whether the proteins are produced using plants or other methods.

However, the production of these proteins in plants in the environment, and in food crops, introduces several unique challenges from regulatory and risk-assessment perspectives. Most of these challenges arise from the simple fact that the plants are being produced in the open environment, a unique aspect in pharmaceutical manufacturing. In a field environment, special containment is possible and amenable to strict regulation, but containment is inherently less certain compared with traditional pharmaceutical manufacturing processes. Because of this, questions of potential intra- and inter-species gene flow, allergen exposure to the public and non-target organism exposure come into play.

Owing to the potential environmental exposure, plant-based pharmaceutical production in the USA necessitates the regulatory involvement of both the USDA and the USFDA (USDA Animal and Plant Health Inspection Service 2003; www.aphis.usda.gov). Specific regulations for producing pharmaceutical proteins in plants are currently being developed, but to date the precise activities and responsibilities of the regulatory bodies within each of the two major agencies are still evolving [5] (USFDA 2002; http://www.fda.gov).

Assessing human and ecological risk from plant-based pharmaceuticals

Regardless of how specific regulatory activities and responsibilities unfold, the human and ecological risks associated with cultivating these plants in the environment must be assessed using the most robust, transparent science-based methods available. In our opinion, the established paradigm of risk assessment offers the best approach for assessing these risks.

Risk assessment has been defined as a formalized basis for the objective evaluation of risk in a manner in which assumptions and uncertainties are clearly considered and presented [6]. The risk-assessment framework that is practiced most frequently today follows the 'Red Book' paradigm, which was put into effect by the United States National Research Council in 1983. Risk assessment flows in a logical, stepwise fashion that includes the following procedures: (i) problem formulation; (ii) hazard identification; (iii) dose–response relationships; (iv) exposure assessment and (v) risk characterization. Hazard and dose are considered in relation to exposure to determine risk or to determine what additional data are needed to calculate or refine risk estimates. For chemical risk assessments in which a chemical, such as a pesticide, is disseminated into the environment, the exposure assessment step is typically crucial for adequately characterizing risk. Conversely, the problem formulation and hazard identification steps are arguably most important when considering risk from plant-based pharmaceuticals.

The problem formulation step establishes the goals, breadth and focus of the assessment. Questions that might be addressed during the problem formulation stage include: (i) what is the stressor or activity causing harm? (ii) what are the potential ecological effects? (iii) what are the potential human health effects? (iv) what are the potential exposure scenarios? (v) what are the potential routes of exposure? The hazard identification step is the act of determining what the hazard is and ascertaining its ability to cause harm.

The answers to these questions and the appropriate actions to take might be self-evident for an environmental contaminant, such as a pesticide, which is designed to kill or deter certain organisms, but they are hardly self-evident for many plants expressing certain pharmaceutical proteins. For example, what is the stressor in a system in which a potato plant expresses a bovine-specific antigen that will be used as an oral veterinary vaccine? Does the recombinant protein have the ability to cause harm to humans or the environment? Is the potato plant itself hazardous? How do we identify the hazard? Should we conduct a battery of toxicity tests on a large group of non-target species irrespective of our knowledge of the specificity of this antigen or the possibility of exposure?

Because of their specificity, lack of toxicity and therapeutic or disease-prevention capabilities, many pharmaceutical proteins that will be produced in plants will challenge our ability to define an environmental hazard. Simply because these proteins are in the environment does not necessarily make them environmental contaminants or human health hazards in the same way we traditionally view chemical stressors. Therefore, imposing a rigid regulatory scheme for these proteins that is similar to the way pesticides are regulated would be a questionable use of finite societal resources.

…imposing a rigid regulatory scheme for these proteins that is similar to the way pesticides are regulated would be a questionable use of finite societal resources.

The staggering variety of recombinant proteins that can be expressed by plants demands that the risks associated

with them be assessed on a case-by-case basis and case-by-case analysis fits well within the stepwise nature of risk assessment. For example, the potential ecological risks associated with potato expression of a bovine-specific antigen and maize expression of a non-specific neutralizing oral antibody to suppress *Campylobacter jejuni* in chickens could be dramatically different. For each case the problem formulation, selection of surrogate species for effects, selection of effects endpoints and selection of exposure routes would not be interchangeable and would therefore need to be evaluated separately.

Risk assessment, communication and management

Despite the limitations discussed here, there are several reasons why the risk assessment paradigm is sufficiently robust to address risk from this technology. Risk assessment is used as a societal decision-making tool for many technologies, at this point including commercialized crops produced using recombinant DNA technology. Risk assessment fits well within the larger paradigm of risk analysis, which also includes risk management and risk communication. All three facets of risk analysis are crucial for effective regulation and policymaking in a democratic society [6,7].

Risk assessment is used as a societal decision-making tool for many technologies, at this point including commercialized crops produced using recombinant DNA technology.

Risk assessment is amenable to both quantitative and qualitative approaches [6]. The ability to describe risk qualitatively will probably be important for plant-based pharmaceutical production because of difficulties in establishing hazards caused by the highly specific proteins that could be expressed. However, the ability to describe these risks quantitatively is probably more important for comprehensive societal decision-making and communication [8,9]. Indeed, the public is more receptive to information presented within an objective, statistical context [10]. Although the complexity of quantitative risk assessment will often make it inaccessible to the layperson, the implementation and communication of these powerful techniques for assessing environmental risks from plant-based pharmaceuticals will help enhance public trust in the decision-making processes surrounding the technology [9].

Technologies are based on science; therefore, science-based frameworks must be used to assess risk from those technologies. To that end, the risk assessment paradigm is sufficiently robust to assess risk from plant-based pharmaceuticals. Although not without limitations, risk assessment is being used at present by all of the US regulatory agencies that are overseeing plants produced using recombinant DNA technology.

To increase public trust in plant-based pharmaceuticals, regulatory agencies should not only employ the risk-assessment paradigm, but also communicate the procedures, risks, and decisions to the public. To our knowledge, risk assessments for pharmaceutical proteins and the transgenic plants that produce them are not being communicated to the public in the USA, but clearly there is a need for a more concerted effort to engage the public.

Although risk assessment should be used to evaluate risk from plant-based pharmaceuticals, we recognize that regulations in a democratic society are rarely made based on risk assessment alone. Rather, risk assessment should form the foundation on which other factors, such as public perceptions, economics, benefits and law must be added before a decision is made. Fortunately, the paradigm of risk analysis is useful because it can be defined as a rational framework whereby the knowledge-based description of risk (a science-driven process) is integrated with social, cultural, economic and political considerations to manage and communicate risk in policy decisions and implementation. Consequently, the paradigm of risk analysis, in which risk assessment resides, is capable of incorporating public perceptions into the decision-making process.

Acknowledgements

We thank A. Fischer, K. Puliafico and M. Rolston (Montana State University) for earlier reviews of the manuscript. This work was supported, in part, by a USDA Special Research Grant to the Institute for Biobased Products, Montana State University, Bozeman, Montana.

References

1 Fischer, R. *et al.* (2003) Production of antibodies in plants and their use for global health. *Vaccine* 21, 820–825
2 Ma, J.K-C. *et al.* (2003) The production of recombinant pharmaceutical proteins in plants. *Nat. Rev. Genet.* 4, 794–805
3 Sala, F. *et al.* (2003) Vaccine antigen production in transgenic plants: strategies, gene constructs and perspectives. *Vaccine* 21, 803–808
4 Fitzgerald, D.A. (2003) Revving up the green express. *The Scientist* 17, 45
5 United States Department of Agriculture, (2003) Field testing of plants engineered to produce pharmaceutical and industrial compounds. *United States Federal Register* 68, 11337–11340
6 Stallones, R.A. ed. National Research Council (1983) *Risk assessment in the federal government: managing the process*, National Academy Press
7 Stern, P.C. and Fineburg H.V., eds, National Research Council (1996) *Understanding Risk: Informing Decisions in a Democratic Society*, National Academy Press
8 Bárdossy, A. and Duckstein, L. eds, (1995) *Fuzzy Rule-Based Modelling with Applications to Geophysical, Biological, and Engineering Systems*, CRC Press
9 Wolt, J.D. and Peterson, R.K.D. (2000) Agricultural biotechnology and societal decision-making: the role of risk analysis. *AgBioForum* 3, 291–298
10 Porter, T.M. (1995) *Trust in numbers: the pursuit of objectivity in science and public life*, Princeton University Press