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Emerging guidelines for the regulation of plant-based drugs are being put into effect by the Pharma-Planta initiative.

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Abstract; Rapid progress in recent years has brought plant-made pharmaceuticals (PMPs) out of the early stages of research and into clinical trials; the first commercial medications for human use are projected to hit the market in 2009. It is yet to be seen if PMP technology will be commercialized in Europe, although at least one product has already entered phase II clinical testing. These new goods pose a threat to the existing, convoluted regulations that control the creation of both genetically modified (GM) plants and "conventional" medicines. Specific rules for the regulation of PMPs are now being drafted, and the areas of responsibility amongst the several EU regulatory bodies are being mapped out. In this post, we'll go through some of the challenges that have come up during the process of developing rigorous risk assessment and risk management methods focused on health and environmental effect, all while cooperating with EU regulatory agencies to provide adequate regulatory monitoring..

Keywords; Medical plants as medicines; PMP; biosafety; regulations

Introduction

The next big commercial breakthrough in biotechnology will probably be the harnessing of plants to create medicinal and industrial proteins. Some advantages of plant-based cell culture systems have been identified over more traditional mammalian and bacterial cell culture methods (Twyman et al. 2005). There are several advantages to mass production, including as inexpensive initial investment, scalability, and storage options, and the possibility of producing large quantities of products at low unit prices. Plant manufacture may be the sole alternative for certain high-demand medicinal goods, especially in underdeveloped nations where they are critically required.

The USDA Centre for Veteri- nary Biologics registered Dow AgroSciences' first plant-derived vaccination in January 2006 (http://www.dowagro.com/animal- health/). The item was a vaccination for chickens.

tobacco cells in sterile, airtight containers to combat Newcastle disease. In validating the safe use of genetically modified plant cells to create therapeutic proteins, this was a major step forward for the industry. Tobacco plant production of the monoclonal antibody CB-Hep.1 was approved by Cuba's Med- ication Quality Control Agency in April 2006. The Cuban Institute of Biotechnology and Genetic Engineering (CIGB) (www.cigb.edu.cu). The production of hepatitis B vaccines requires this specific monoclonal antibody. The contemporary pharmaceutical business and authorities may be more accustomed with biore- cycling settings, but this product is the first commercial use of complete plants as a production vehicle for reagents utilized in a clinical manufacturing procedure.

Although some devices have progressed to the clinical trials stage, PMP technology has not yet been used commercially in Europe. Cobento Biotech produces human intrinsic factor in Arabidopsis thaliana, while Meristem Therapeutics produces gastric lipase and lactoferrin in maize (www.meristem-therapeutics.com) (www.cobento.dk). Several organizations are now responsible for regulating these crops; which organizations are involved will vary on factors such as the host plant used, the location of cultivation, the product selected, the final formulation employed, and the population for whom the crop is intended.

Pharma-Planta

In 2004, the European Union (EU) established an academic research group called Pharma-Planta (www.pharma-planta.org) to address many issues related to the use of plants as manufacturing platforms for medicines. One of the consortium's goals is to establish a plant-based manufacturing platform for medicines suitable for the European market, and another is to aid in the creation of suitable regulatory control in the EU.

The second goal is to create a system for manufacturing transgenic plants that can make recombinant antibodies in accordance with established guidelines for doing so, known as Good Manufactur-

Human Phase I Clinical Trials, Pre-Clinical Toxicology Testing, and Good Manufacturing Practice (GMP) in Europe.
Third, to show concrete support for the humanitarian use of PMPs and the related industrial technologies in low-income nations. A unique consortium-wide Statement of Intent on the use of Pharma- Planta intellectual property for humanitarian purposes in underdeveloped

stringent regulatory restrictions pertaining to other GM crops, such as the 2001/18 EU laws and the USDA/APHIS permit application criteria for the USA (for field grown plants), but also the regulations posed by authorities that regulate the manufacturing of pharmaceuticals. In 2002, both the FDA (US Food and Drug Administration, URL: http://www.fda.gov/cber/gdlns/bioplant.pdf) and the EMEA (The European Agency for the

countries and the establishment of a PMP licensing approach for humanitarian reasons have enabled this.
To define the most up-to-date ideas on appropriate international regulatory oversight and the benefits and drawbacks of using various plant species for the production of recombinant pharmaceuticals, a Pharma-Planta sub-group responsible for the analysis of biosafety issues conducted a consultation exercise. Recent years have seen a proliferation of reviews devoted to this same subject (Commandeur et al. 2003; Mascia and Flavell 2004; Petersen and Arntzen 2004; Ma et al. 2005a, b). The purpose of this study is not to repeat the information found in these other works, but rather to examine the more general implications involved in the development of recombinant pharmaceuticals in plants. We talk about picking products, production hosts, and ideal growing conditions. Choosing the proper product is crucial for the industry to assure the success of this new technology and its adoption by regulators.

Where do we go from here?

Many technological and regulatory considerations must be addressed for PMP production to be effective (Horn et al. 2004; Ma et al. 2003). How well the target product can be produced, constructed, and stored in the host plants, as well as how effectively it can be removed, all play a role (Gomord 2004; Tekoah 2004). The pharmaceutical product yield per hectare that can be achieved, the cost of inputs, harvesting, transportation, and processing, and the cost of marketing are all factors to consider while deciding on a production host (Fischer et al. 2004; Stoger et al. 2005; Giddings et al. 2000). Regulatory agencies have a number of requirements that the production system must meet.

Evalua- tion of Medicinal Products, URL: http://www.
emea.eu.int/pdfs/human/bwp/076402en.pdf)
published draft documents addressing quality aspects of the production of medicinal products in GM plants.
Ability to induce enough expression and accumulation of the recombinant protein in plants is a critical factor in determining economic

viability. In order to fully take use of plants' scalability in agriculture, this is an essential first step (Ma et al. 2003; Twyman et al. 2003; Hood et al. 2002). Although the plant species utilized in production has some bearing on absolute yields, there are many other variables that have a role in determining which crop is selected for cultivation. What are the pros and downsides of various crop production systems? What are the most pressing biosafety and regulatory concerns? These were some of the primary topics discussed throughout the consultation.

Production Method Selection

The production host's biological characteristics must be evaluated from several angles, including productivity, environmental impact, food safety, and human health. There is probably not a single plant species that can meet all the needs. In order to choose the optimum species for a certain application, it is sometimes necessary to strike a compromise between competing factors. There are three possible "classes" of plant species to utilize: wild species, crops that are not consumed by humans, and crops that are consumed by humans.

Species that aren't bred in captivity

The term "non-cultivated species" is used to describe the wide variety of plant life that is not cultivated by humans. Like

The main benefit of non-food crops is that they are not included in the human food chain. Conversely, little is understood about the genetics and biology of such organisms, including whether or not they generate poisons and whether or not they are capable of outcrossing. To make field farming more possible, little to no effort will have been made to domesticate such species. Due to a lack of domestication, seed yields are frequently poor, making leaf material the most probable harvestable target tissue.

The cultivation of non-domesticated plant species in bioreactors or other forms of confinement is theoretically viable. According to Biolex (http://www.biolex.com/), who bought Lemnagene (http://www.lemnagene.com) in 2005, duckweed has a high potential for scalability (Lemna minor). One may also argue that raising this species in captivity has its benefits.

It's quite improbable that we'll be able to successfully domesticate a creature that isn't already part of human culture anytime soon. Although it may be possible to create new species of "pharmaceutical crop" in the long run, the appropriate methods should be assessed in tandem with studies to use already domesticated species rather than in place of them.

(i) Non-food crops

The main advantage of non-food crops is that, although they have been developed and bred as crops, they are not used for food or feed. Consequently, it should be relatively easy to keep them separate from crop products used in the human or animal food chain. The main species being considered in the non-food category are tobacco and falseflax. Tobacco is a strong candidate for the commercial production of recombinant proteins since it already has a track record in PMP research (Stoger et al. 2002) and has recently been used in Cuba for the commercial production of a recombinant anti- body against hepatitis B (Ramirez et al. 2003; Valdés et al. 2003; Pujol et al. 2005Principal tobacco benefits infrastructure set up for massive processing that works properly. Unless it is produced in rotation with a food crop, it is quite improbable that tobacco material will accidentally mix with material intended for the human or animal food chain (cf. the ProdiGene maize incident, discussed later). These problems should be resolved when Good Agricultural Practice becomes more well-developed.

Toxic alkaloids are produced in high concentrations by many tobacco cultivars and must be eliminated during processing, however there are low-alkaloid types that might be used to create medicines (Fischer and Emans 2000; Ma et al. 2003). Alternatively these alka- loids are allegedly not present in cell suspension cultures, which might also be utilized to create recombinant proteins (Doran, 2000; Hellwig et al. 2004), but not on the scale necessary for the antibodies selected as target molecules in the Pharma-Planta initiative. Alternatively, proteins might be sent along the secretory route and then secreted by the plant at the root or leaf level (Drake et al. 2003; Kormarnytsky et al. 2000; Borisjuk et al. 1999). There are phenolic compounds in tobacco that are produced during grinding and protein extraction that may impede further processing steps. However, developments in downstream processing, such as the use of smart membranes during the clarifica- tion and capture stages, will

potentially enable manufacturers to target and eliminate these undesirable molecules, making them no more a problem than the removal of any other protein in the purification process.

The Finnish Biotech Company UniCrop (www.unicrop.fi) is developing falseflax (Camelina sativa) to manufacture recombinant proteins for the pharmaceutical business. Protein is recovered from the soft sprout material generated from transgenic seedlings in fully contained air-lift bioreactors, eliminating the need to separate the fibres and oil later in the processing chain.

(ii) Food crops

Cultivating food crops and undergoing transformation operations are accompanied by a wealth of information. also clear cut for a few key agricultural species. The regulatory benefits of using GRAS (Generally Recognized as Safe) plants are not to be overlooked. EMEA draft guidance paper on PMPs, www.emea.eu.int/pdfs/ human/bwp/076402en.pdf, emphasizes that GRAS status only considers oral administration and does not apply to topical or injectable forms.
Seed crops (a), vegetable crops (b), and fruit/green leaf crops (c) are the three main categories of food crops. The primary distinction between the three classes is the amount of time that passes after harvest before the plant tissue holding the medicinal component must be preserved in some other way, such as by desiccation or freezing

Regulation of plants for pharmaceutical production (US and EU)

Most of our expertise in regulating the field release of pharmaceutical plants has been acquired in North America. European Union officials are making an effort to treat pharmaceutical crops the same way they treat other agricultural crops—on a case-by-case basis. However, most regulatory systems do not conveniently accept pharmaceutical crops since the laws have mostly been devel- Developed for use in food and feed crops, with consideration given to any possible environmental consequences. Although some effort has been made to modify these rules to accommodate pharmaceutical crops, the European Union regulatory process currently provides no "natural home" for conducting such an evaluation. Currently, under 2001/18/EC, the responsible authority in the country of release must be notified of every field-grown pharmaceutical crop growing inside the EU. Both food and non-food crops have their release plans governed by these rules and regulations. In accordance with regulation 1829/2003/EU, the European Food Safety Authority (EFSA) would evaluate a request for commercial distribution of a food crop. EFSA would also have the determining responsibility if a non-food crop was proposed, but would normally only intervene in instances where Member States were not in agreement. Currently, EFSA is working on a set of guidelines geared specifically for PMPs. Note that the field release restrictions would not apply to a pharmaceutical crop cultivated in containment; instead, the confined regulations (Directive 90/219/EEC as revised by Directive 98/81/EC) would govern. Whether cultivated in confinement or not, medicinal products from plants would also need to conform to the 2309/93/EU standards. During the early stages of clinical trials, the relevant national authority is in charge of these rules, but at the point of commercial application, the European Medicines Evaluation Agency (EMEA), which is roughly comparable to the FDA in the US, takes control. In 2002, the EMEA released some preliminary recommendations that are now being revised (http://www.emea.eu.int/pdfs/hu-man/bwp/076402en.pdf) (publication due by the end of 2006). The biological and semantic distinctions between plant-based manufacturing and traditional systems based on cells grown in bioreactors have contributed to the delay in finalizing these guideline notes. Concepts like working and master bank stocks, batch-to-batch consistency, standard operating procedures, and so on all need to be specified precisely for plants (inputs, downstream processing, QA etc). It is still being determined at what point in the process each regulatory authority gets engaged and the scope of their power. Field-release A permit from APHIS is needed for PMP crops planted in the US (the Animal and Plant Health Inspection Service of the USDA). A containment strategy for growing, harvesting, and transporting plants from the field is required. Seed production, pollination

schedules, harvest dates, crop destruction, shipping, quarantine, and storage and usage of equipment are all subject to APHIS scrutiny. Up to five inspections of the fields may be carried out during the growing season, each time coinciding with a crucial stage of harvest. Field test permits are issued by APHIS to organizations like companies and universities, who then may subcontract with individuals like farmers. Training on the necessary permits and their implementation is mandatory for subcontractors (Elbehri 2005).

There are no plans for APHIS (USDA) to deregulate any medicinal crops at this time. That's why it's probable that commercial and research crops alike will continue to need an experimental permit and the extra scrutiny that comes with it. The possibility for accidental cross-pollination between pharmaceutical and food crops is of special concern to the FDA. Attitudes on the use of food crops to create "drugs" have been profoundly impacted by the Star Link maize issue (while not a PMP crop), in which GM maize intended for animal feed, invaded the food chain. The USDA has the right to mandate environmental impact statements for any permit applications they deem necessary (EA). Such EAs might be triggered by factors like the proposed site's location or the projected cultivation conditions. The United States Department of Agriculture (USDA) has published two separate Environmental Assessments (EAs) for Prodi- Gene's maize field experiments (Permits 04-121- 01r and 04-114-01r, available at http://www.aphis.usda.gov/brs/ ea pubs.html). These materials were made available for public review, and the deadline for submission of comments was extended (from the standard 30 days). Subsequently, we decided to stop considering the applications.

International developments in regulation

Canada's Food Inspection Agency (CFIA) held a technical conference for researchers from across the world in 2004.
shop on how PMP products and by-products should be separated and handled in commercial settings. Experts in grain handling and identity preservation were among the participants, along with members from the PMP sector, federal government organizations, agriculture and agribusiness organisations, and more. Since many of the plants being researched for PMP production are also used as food and feed (such as safflower and alfalfa), the first step in formulating a regulatory framework was to investigate whether PMP products and by-products could be adequately segregated from other commodities, and more specifically from commodities intended for the food and feed chains. The workshop's findings are available online at www.inspection.gc.ca/english/plaveg/bio/mf/ segrege.shtml on the CFIA site.

In 2004, APHIS held a similar international workshop titled "Confinement of Genetically Engineered Crops During Field Testing." The primary objective of this session was to analyze data from existing crop plants that have been planted with APHIS field trial permits to create PMPs and plant produced industrials (PMIs). Summaries of the many environmental consequences and confinement concerns discussed during the workshop may be accessed on the APHIS website (www.aphis.usda.gov/brs/confine workshop 2004. html).

The European Food Safety Authority (EFSA) and the European Medicines Agency (EMEA) are both working on guidance notes for the regulation of PMPs in Europe. As items are put through the new regulatory procedures, these rules are certain to change further..

There is an unexpected occurrence of pharmaceutical crops in food crops.

Gens encoding pharmaceuticals may be difficult to contain. Both the spread of genes and the appearance of volunteer plants in successive harvests pose serious risks to both ecosystem health and human wellbeing. The attention and care given to this problem is reflected in the activities taken by regulatory bodies and the biotechnology industry in the past. Prod- iGene Inc. was at the epicenter of a 2002 controversy about how to keep pharmaceutical crops grown outdoors from spreading (Hoag 2003). Volunteer transgenic maize plants appeared in the soybean crop the year after the pharmaceutical maize crop, and the incident received widespread media attention. Soybean in the storage silo was seized and destroyed because of the presence of maize plant debris.

As part of the subsequent settlement, Prodigene agreed to pay $250,000 in civil penalties, the expense of cleaning the premises and equipment, and the price of 500,000 bushels of soybeans. While this instance reveals that mechanisms were in place to prevent tainted foods from entering the food supply, it also highlights that the biology of the production host crop and subsequent crops in the cycle must be taken into consideration for effective regulation and containment. While this should help lessen the possibility of contamination, it may be unrealistic to promise that it will be eliminated entirely. According to Elbehri (2005), a coalition of food companies favored the inclusion of a food-safety review per event prior to obtaining a permit. In reality, this might shift the focus of agricultural research and development away from staple crops like maize and toward non-food crops (tobacco).

Transparency

Many forms of regulation need openness to the public. Generally speaking, releasing GMOs into the environment needs authorization from the appropriate authorities in nations with biosafety legislation. In nations that are not yet regulated for biosafety but are signatories to the Cartagena Protocol on Biosafety (CPB) (http://www.biodiv.org/biosafety/default.aspx), notice is required before any genetically modified organisms (GMOs) are moved across borders for release into the environment. When a product is released into the environment in the European Union (EU), either as an experimental release (2001/18/EC part B) or as a commercial release (2001/18EU part C) (http://gmoinfo.jrc.it/), the EU system requires that a summary of the notifier's application and the assessment report be made available to the public. When it comes to government agencies in the United States, some (like the USDA) make their applications and notices available to the public, while others (like the FDA) only do so upon request. According to South Africa's GMO Act, information on the GMO's description, the release's intended purpose and location, the release's monitoring, and the evaluation of its environmental effects "must not be kept hidden."

Strategies for reducing the impact

Low-tech methods, such as careful planning and execution of each operation, are primarily what is needed to prevent pharmaceutical crops from entering the food chain. To prevent the introduction of the industrial characteristic into traditional breeding stock, the crop must be cultivated in isolation from breeding materials. Given the difficulty in detecting such mixture in reality, proper rules for handling and labeling are crucial. Similarly, traditional agricultural crop experiments must be conducted separately from both local and large-scale field trials. To prevent accidental pollination from occurring, commercially produced parent seed and commercial crops must be cultivated in isolation from other plants of the same species or wild relatives.

For plants that rely on either wind or insects for pollination, achieving an adequate amount of isolation may be a significant challenge. In all likelihood, the new crop shouldn't be produced in areas where it may come into contact with food crops or related wild weeds. places where the species is often cultivated for human consumption, or where it is abundant in the wild.

The qualities of the chemical, the biology of the crop, and the nature of the environment in which it is cultivated are only a few examples of the variables that will determine the most effective mitigation methods for a certain pharmaceutical crop. Here are a few of the recommended preventative measures: (reviewed by Commandeur et al. 2003; Dunwell 2005).

• Using marker genes to make the crop or its products (such as seeds) physically distinguishable from food and feed crops, such as DsRed (Disco- soma sp. red fluorescent protein; www.clon- tech.com/).

Injecting the crop with a bitter or unpleasant flavor to make it unattractive; Expressing the crop after harvest; Keeping the crop at a safe distance from sexually compatible crops, weeds, and feral species; Using barrier crops to reduce cross pollination

• Geographical and temporal separation to prevent genetic mixing of crops. The risk of cross-pollination may be reduced by sowing crops at intervals of time other than when they are being harvested.

Pollination may be avoided by physically removing flowers from a garden. In order to prevent pollen from spreading from the female transgenic parent, tassel removal is a common isolation method in maize.

Agricultural crops and their products should not come into contact with PMP crops, hence precautions should be taken to prevent this. Measures to prevent the negative consequences of volunteer plants growing in subsequent years; partial processing of the pharmaceutical product at the production site; secure land with security fencing; dedicated agricultural machinery; dedicated storage facilities; secure methods of transporting seeds for establishing the crop; and secure methods of transporting the pharmaceutical containing crop product (such as seeds) and crop residues.

Conclusions

While there are certainly advantages to using maize in pharmaceutical manufacture, numerous biotechnology firms have looked at the viability of using a variety of crops, and thus far, no one crop has stood out as the obvious winner. The preferences of one company may differ from another's based on the specifics of its business strategy. The adoption of a food crop is likely to have the greatest impact on the social and political factors that drive crop selection. It would seem that maize's numerous benefits in the short-term include its familiarity, infrastructure, scalability, product stability, and processing simplicity. The introduction of alternative crops has the potential to streamline production and improve public image over the long run. If genetic modification (GM) technology is to reach its full potential, it is imperative that it get widespread public support. Thus, it is important to thoroughly examine both food crops and non-food crops.

The anti-HIV monoclonal antibody production under the Pharma-Planta initiative will center on maize. The product (a topical cream) has entered phase 1 clinical testing, and the crop will be cultivated in a controlled environment. The potential of tobacco as a supplementary crop is being studied. By addressing many of the issues raised in this article, it is intended that this program would serve as a beta test for the emerging regulatory requirements for PMPs.

There is a substantial possibility of a demonstrable public benefit from pharmaceutical crops. Any mistakes made with pharmaceutical crops might represent a big setback for their future implementation, especially in light of the existing bad perceptions surrounding GM crops. Any manufacturing system that has the potential to provide universal access to medications, especially in countries with substantial poverty, deserves careful consideration for the value it might provide to mankind.

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