資料 4

PDA 2nd Monoclonal Antibodies Workshop
QbD: Science to Submission Approaches
Munich | 25 - 26 June 2009
Rev: 23 Feb 09

"AGENDA AT A GLANCE"
(Breaks and ample discussion periods not shown)

Thursday, 25 June

9:00 Welcome

Implementing QbD for Monoclonal Antibodies: Science and Risk-based Strategies
The morning and after-lunch sessions will review two industry case studies demonstrating how QbD principles can be put into practice in the development of monoclonal antibody products. The presentations focus on scientific considerations and emphasize approaches and tools generally applicable to the QbD concept.

- Case Study 1: QbD Development of a Monoclonal Antibody : Outcomes of the FDA / Conformia Cooperative Research and Develoment Agreement (CRADA), 'Pharmaceutical Development Study.'

12:00 Lunch

- Case Study 2: QbD for Cell Culture Design : Experience of F. Hoffmann LaRoche, Penzburg

Regulatory Session : European Regulatory Perspectives on Quality by Design
The recently published EMEA Guideline on production and QC of monoclonal antibodies outlined current regulatory thinking on the development, production, characterization and specifications for monoclonal antibodies and related products. These presentations, by leading regulatory experts in the forefront of EMEA thinking, offer perspectives on the guideline and its relationship to QbD principles.

- QbD – General principles
- QbD – Issues Related to MAb

17:00 End of day, Networking Event

Friday, 26 June

8:30 Morning Sessions : The Science of QbD: Strategies, Enabling Technologies and Monograph Considerations
This half day provides deeper technical perspectives on the scientific strategies being utilized for developing monoclonal antibodies following QbD principles. Presentations will focus on the important issue of oligosaccharide profiling and other analytical technologies for enabling QbD.

- Analysis of Critical Features of Antibody Glycosylation and their Roles in Modulating IgG Effector Function
- A Quality by Design (QbD) Approach to Monoclonal Antibody Glycosylation
- Industry Perspective on the Proposed Changes to Monograph 2031
- EDQM Rationales for Proposed Changes to Monograph 2031
- Analytical Technologies for QbD and Control Strategies

12:00 Close of Workshop

Review

Evolution of a regulatory framework for pharmaceuticals derived from genetically modified plants

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The use of genetically modified (GM) plants to synthesize proteins that are subsequently processed, regulated and sold as pharmaceuticals challenges two very different established regulatory frameworks, one concerning GM plants and the other covering the development of biotechnology-derived drugs. Within these regulatory systems, specific regulations and guidelines for plant-made pharmaceuticals (PMPs) – also referred to as plant-derived pharmaceuticals (PDPs) – are still evolving. The products nearing commercial viability will ultimately help to road test and fine-tune these regulations, and might help to reduce regulatory uncertainties. In this review, we summarize the current state of regulations in different countries, discuss recent changes and highlight the need for further regulatory development in this burgeoning, new industry. We also make the case for the harmonization of international regulations.

Introduction

The production of pharmaceutical proteins in plants has several potential advantages over current systems such as mammalian and bacterial cell cultures, including the lower costs and scalability of agricultural production, and the absence of human pathogens [1,2]. A large number of plant host systems has been tested, including plant cell cultures, unicellular plants, aquatic plants grown in containment, and, most notably, food and non-food crops, which can be grown in greenhouses, underground growth facilities, or the open field [3].

Research and development in the area of plant-made pharmaceuticals (PMPs) over the past 10 years has focused on agricultural crops, with tobacco, maize, potato, rice and safflower being the most frequently used. However, regulatory uncertainty and technical challenges in downstream processing [4] have prompted the development of PMPs produced in contained systems, such as plant suspension cells [5] (e.g. a carrot cell system developed by Protalix) and the *Lemna* system, as championed by Biolex Therapeutics. Products in these systems have reached phase III and phase II clinical trials, respectively [6]. In 2006, the United States Department of Agriculture (USDA) licensed a poultry vaccine produced in cultured tobacco cells [7]. Since then, several products derived from crop plants have also reached late development stages, including human insulin and carp growth hormone produced in safflower. These are expected to reach the market between 2008 and 2010 (see Table 1).

PMPs present two major challenges for the regulatory bodies. Regulators of agricultural biotechnology are confronted with a novel type of crop use, and drug regulators must deal with a novel drug-production concept. Particular challenges arise in the case of open-field production, in which more than 350 field trials have been approved for crops producing either pharmaceutical or other industrial proteins in the USA, Canada and the European Union (EU) over the past two decades [8]. The USA and Canada have published several discussion papers and drafted PMP-specific guidelines [9–15], yet these guidelines have not been finalized and will probably evolve further with technological developments.

Here, we provide an overview of the regulations governing the cultivation of pharmaceutical plants and the approval of PMP products. We focus on PMPs produced by agricultural cultivation, because these pose a greater regulatory challenge than contained production systems. Non-pharmaceutical products (i.e. plant-made industrials [PMIs]) are outside of the scope of this review. We first set out the requirement for specific regulations and guidance, and then describe the most recent regulatory developments for pharmaceutical plants and the licensing of PMPs at both the national and international levels. We conclude with a discussion of remaining regulatory challenges. A list of relevant websites is provided in Box 1.

Why do we need specific regulations for PMPs?

Regulatory oversight of genetically modified plants

Several differences have been drawn among first-, second- and third-generation genetically modified (GM) crops. First-generation crops have traits such as herbicide tolerance and insect resistance, second-generation crops have improved food and/or feed (hereafter food/feed) quality, and

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Table 1. Plant-made pharmaceuticals in advanced stages of development[a]

Product	Application	Plant host(s)	Status [b]	Company or academic group
Plant-made pharmaceuticals and vaccines – human use				
AB	AB cancer vaccine	Tobacco	Phase II clinical trials	Large Scale Biology, USA [c]
B subunit of heat labile Escherichia coli toxin LT-B	Oral vaccine against traveller's diarrhoea	Potato, maize	Phase I completed (potato: 1998) [d]	[89]
Capsid protein Norwalk virus	Vaccine	Potato	Phase I completed (2000) [d]	[90]
CaroRx™ [e]	AB carries prophylaxis	Tobacco	Phase II clinical trials, approved as medical device in the EU in 2003	Planet Biotechnology, USA
DoxoRx™ antibody	Side-effects of cancer therapy	Tobacco	Phase I completed	Planet Biotechnology, USA
Fusion protein, including epitopes from rabies	Vaccine against rabies	Spinach (virus-infected)	Phase I completed [d]	[88]
Gastric lipase	Cystic fibrosis	Maize	Phase II clinical trials, commercialisation expected for 2009/2010	Meristem Therapeutics, France
Hepatitis antigen	Oral vaccine against hepatitis B	Potato	Phase II clinical trials	Azbio, Arizona State University, USA
Human glucocerebrosidase (prGCD),	Treatment of Gaucher's disease	Carrot suspension cells	Received FDA approval for Phase II clinical trial of prGCD; marketing expected in early 2008	Protalix Biotherapeutics, Israel
Insulin	Diabetes	Safflower	Path for clinical trials accepted by FDA, commercialisation expected for 2010	SemBioSys, Canada
Lactoferon™ (α interferon)	Hepatitis C	Lemna	Phase II	Biolex, USA
RhinoRx™ [e]	Common cold caused by rhinoviruses	Tobacco	Phase I/II planned for 2005 [d]	Planet Biotechnology, USA
Plant-made pharmaceuticals and vaccines – animal use				
Antigen	Vaccine against feline parvovirus	Tobacco	Advanced	Large Scale Biology, USA [c]
Antigen	Vaccines against papilloma virus	Tobacco	Early	Large Scale Biology, USA [c]
HN protein of Newcastle disease virus	Poultry vaccine	Tobacco suspension cells	Approved by USDA	Dow Agro Sciences, USA
Plant-made pharmaceuticals applied as nutraceuticals				
Human intrinsic factor	Food supplement, vitamin B12 deficiency	Arabidopsis	Approval from Danish authorities for commercial production in greenhouse; market authorisation in Poland	Cobento Biotech AS, Denmark
Human lactoferrin	Developed as food supplement: anti-infection, anti-inflammatory and iron-binding properties	Rice	Advanced [f]	Ventria, USA
Human lysozyme	Developed as food supplement: anti-infection, anti-inflammatory and iron-binding properties	Rice	Advanced [f]	Ventria, USA
Immunosphere™ [g]	Carp somatotropin to be used as feed additive for shrimps	Safflower	Only import permits required for USA, Canada or the EU; commercialisation expected for 2008	SemBioSys, Canada

Information from references [16,89–91], updated and expanded from company websites and literature. Colour code: orange, open field production; green, greenhouse; blue, entirely contained (cell culture, bioreactor-type) production; no colour, production environment unknown. Abbreviations: AB, antibody; EU, Europe; FDA, Food and Drug Administration; HN, hemagglutinin/neuraminidase; LT-B, labile toxin B-subunit; prGCD, plant-cell recombinant glucocerebrosidase; USDA, US Department of Agriculture.

[a] This table cannot be considered a comprehensive list and does also not include PMPs and PMVs that are still in very early phase of development.

[b] For human biopharmaceuticals: phase of clinical trials.

[c] Large Scale Biology filed bankruptcy in 2006.

[d] No updated information available.

[e] Produced from both open fields and greenhouses. Clinical materials have been derived from greenhouses (E. Fineman, personal communication).

[f] Already commercially available as fine chemical.

[g] According to company officials, the carp growth hormone will be used in major shrimp producing countries only (e.g. South America, China, Thailand) and has to seek market authorisation as a food additive in these countries only.

third-generation crops produce added-value products, and thus include PMP crops. First- and second-generation GM crops are mainly intended for food/feed purposes whereas third-generation crops are envisaged as production vehicles for high-value molecules and are not intended for consumption as food/feed. PMP crops are designed to maximize the yield of the target protein, which consequently can accumulate up to 5000 times the level typically found for transgene products in first- and second-generation crops [16]. PMP crops can also undergo

Box 1. Useful websites concerning the regulation of pharmaceutical plants and their products

USA
- USDA-APHIS: www.aphis.usda.gov/
- US permits for pharmaceutical plants: http://www.aphis.usda.gov/brs/ph_permits.html
- FDA CFSAN: http://www.cfsan.fda.gov/
- EPA: http://www.epa.gov/
- US Excellence through Stewardship Initiative: http://www.excellencethroughstewardship.org/
- Biotechnology Industry Association BIO: http://bio.org/healthcare/pmp/

Canada
- CFIA Plant Biosafety Office: http://www.inspection.gc.ca/english/plaveg/bio/pbobbve.shtml; http://www.inspection.gc.ca/english/plaveg/bio/mf/fracad/commere.shtml#3; http://www.inspection.gc.ca/english/plaveg/bio/mf/sumpnte.shtml
- CFIA Feed Section: http://www.inspection.gc.ca/english/anima/feebet/feebete.shtml
- HC: http://www.hc-sc.gc.ca

Europe
- EFSA: http://www.efsa.europa.eu
- EMEA: http://www.emea.europa.eu

International
- Cartagena Biosafety Protocol: http://www.cbd.int/biosafety/

multiple genetic modifications (i.e. stacking) to co-introduce pest resistance, molecular confinement, changes in glycosylation, and identity preservation traits [17–20]. These multiple modifications can also increase the likelihood of unintended effects on the plant [16]. Furthermore, the pharmaceutically active products are designed to elicit a physiological response in humans, and so inadvertently exposing humans or animals to such plant material is generally perceived as a greater concern than the corresponding risk associated with first- and second-generation crops. Pharmaceutical plants are therefore considered to pose additional environmental and health risks, although the actual risk could differ greatly, depending on the properties and expression level of the protein, the nature of the host plant, and the particular exposure scenarios [16,21–26].

The main concerns raised in stakeholder consultations and crucial reports from consumer and environmental organizations are the risk of contaminating the food/feed chain, and broader environmental impacts, including effects on wildlife [27–34]. Even if the actual risks are negligible, farmers and the food industry are concerned about the economic risks should PMP crop residues appear in food products [35–43]. These concerns are also reflected by the USDA policy of zero tolerance, the history of which is discussed in Box 2. However, the adventitious presence of PMPs in food is probably much less likely than contamination with first-generation GM crops, partly because PMP crops will be restricted to relatively small plots of land. For example, ~15 000 acres of PMP safflower could deliver the entire predicted global demand for insulin in 2012 [44]. The absence of a trade in seeds and viable plants, along with maintaining strictly separated processing streams, should further reduce the risk of food chain contamination.

Box 2. The ProdiGene case – a trigger for the USDA's zero tolerance policy

In 2002, the biotechnology company ProdiGene Inc. was fined US$250 000 by the USDA and compelled to carry out a US$3 million clean-up operation after volunteer* maize plants containing the gene for a veterinary vaccine were found among a soybean crop planted in the same field in the following season. Part of the clean-up process included the purchase and destruction of more than half a million bushels of adulterated soybeans, and ProdiGene was also ordered to post a US$1 million bond to fund the development of a compliance programme for future PMP crops.

The ProdiGene case, along with similar incidents involving first-generation GM crops in food products, prompted a robust response by the regulatory agencies; the penalty issued against ProdiGene was the maximum possible under the 2000 Plant Protection Act. This reflected the perceived risk associated with accidental consumption of a pharmaceutical product, and it resulted in a 'zero tolerance' approach to enforcement in which no attempt was made to make penalties proportional to the risk involved. However, it was never shown that the volunteer maize plants were transgenic, or that they produced viable seed containing the vaccine. Nor was there evidence of actual risk. Partly as a result of the controversy over this decision, APHIS envisages moving towards a tiered approach based on the actual risks posed [83].

* A cultivated plant growing from self-sown or accidentally dropped seed.

North American regulators and the biotechnology industry therefore consider pharmaceuticals as a distinct category of GM crops with handling requirements that differ from those required for crops producing food/feed [45]. Existing regulations and guidance documents are considered to be inadequate to govern the commercialization of PMPs and have therefore created regulatory uncertainties for developers. Key elements of proposed regulations and guidance include dedicated machineries and facilities, contract farming, standard operating procedures for many steps of on-farm work, and training programmes for workers (see Box 3). The higher value and lower acreages associated with pharmaceutical crops could make extensive and redundant confinement measures economically feasible. Emerging regulations focus on extensive physical and organizational confinement measures to avoid outcrossing, spillage of seeds or biomass, and co-mingling with food/feed crops [12–14].

PMPs produced in greenhouses and fully contained facilities, such as cell culture systems, fall under different regulations to those governing field-grown crops, and regulations need to be much less stringent as long as containment is maintained. One issue that remains to be dealt with is the level of containment needed. Even within the EU, implementation of GM organism (GMO) legislation at the national level has led to differences in interpretation. For example, GM crops grown in net houses – greenhouses comprising fine-meshed nets instead of glass – are considered as being 'contained' in some EU Member States and as an environmental release in others, with the latter requiring a much more comprehensive dataset for authorization [46].

Regulation of pharmaceuticals

The drug regulators have repeatedly stated that existing guidelines, in principle, also apply to PMPs [13,47–49].

Box 3. Permit conditions for growing pharma plants in the USA[1]

- Separation from sexually compatible crops (e.g. one mile for open pollinating maize)
- 50 feet fallow zone surrounding the plot
- No planting of food/feed crops on the test site in the following year
- Dedicated equipment (not for use with food/feed crops)
- Submission of Standard Operating Procedures (SOPs) required, depending on the assigned risk category for the following:
  - Harvesters and planters
  - Storage facilities for seed and equipment used to handle regulated articles
  - Seed cleaning, processing and drying
  - Equipment to off-load, haul or move seed or harvested materials
  - Tractors including attachments
- Monitoring of volunteers during and after completion of field trials
- Growers under contract with the manufacturers only. Annual APHIS training; approval of training programmes for personnel
- Audit of field trial records by APHIS
- On-site inspections by APHIS at least seven times a year before, during and after production

Sourced from the following references [14,45,88].

[1] This box includes examples of specific requirements for confinement measures of pharmaceutical and industrial plants. For full details see the following references [13,14]. Measures depend on host plant, type of protein, location of production and plant handling practices.

However, it is difficult to follow such guidelines to the letter, because they have been developed for cell-based systems, which are sterile and contained processes in which the media and environment can be controlled precisely. By contrast, whole plants are not sterile and are not necessarily contained, and their environment can be variable owing to the weather, soil heterogeneity, and interactions with other organisms, including pests. Cell-based and fermenter-specific terminologies are also difficult to apply to whole plants; for example, the concept of master and working cell banks. For pharmaceuticals produced in mammalian cells, a master cell bank is an archived frozen stock of cells that can be used to replenish a working cell bank, from which the production cells are derived. Given that plants cannot be frozen like cells, it is impossible to apply the same principles to plant-based systems. Another process that is more relevant to pharmaceuticals derived from mammalian cell lines is virus clearance and inactivation, because mammalian cells can support the replication of human pathogens. This is another potential advantage of PMPs, in that such contamination is of little or no relevance to plants, especially in the case of greenhouse-based production. For field-produced PMPs, the only conceivable – albeit still disputed – source of such contamination would be from rodents, birds and workers. Plant viruses, by contrast, are more likely to be present but are not known to present health risks to humans. Nevertheless, regulators have yet to express their views on this.

Regulations governing the cultivation of PMP crops

Specific regulations and guidance documents for the cultivation of PMP crops have been drafted in jurisdictions with significant commercial research and development (R&D) activity, but not in other areas. This is indicated by the number of field trials that have been approved: 240 in the USA, 90 in Canada, and ~30 in the EU [8]. The development of specific regulations in the USA was largely triggered by a series of compliance failures concerning food/feed GM crops, and – in one case – a PMP crop, which increased public pressure (see also Box 2).

R&D activities have also been tracked in South Africa and Australia, but this has not yet resulted in visible regulatory activities. Some PMP-related commercial R&D is also being conducted in other countries (e.g. South Korea, Japan, China, Chile and Cuba), but little regulatory information is available in the public domain. On an international level, PMP crops have, to date, been taken up only in the context of the Cartagena Protocol on Biosafety (CPB).

USA

The USDA and the Food and Drug Administration (FDA) share responsibility for the cultivation of PMP crops in the USA (Table 2). Within the USDA, the Animal and Plant Health Inspection Service (APHIS) oversees and regulates the release of GM plants into the environment, and also

Table 2. Statutory authorities, regulations and guidance relevant to growing pharmaceutical crops in the US, Canada and Europe

Country	Authority	Scope of regulation	Laws and regulations	Specific regulations and guidance for pharmaceutical crops
	USDA-APHIS Biotechnology Regulatory Services (BRS) www.aphis.usda.gov/	Development and field production from seed through to grain. Including transport and environmental release	Plant Protection Act (PPA). National Environmental Protection Act (NEPA)	Field Testing of Plants Engineered To Produce Pharmaceutical and Industrial Compounds [88]. Introductions of Plants Genetically Engineered to Produce Industrial Compounds (Interim rule) [50] Draft Guidance for Industry: Drugs, Biologics, and Medical Devices derived from Bioengineered Plants for Use in Humans and Animals [13][a]. Draft Guidance for APHIS Permits for Field Testing or Movement of Organisms with Pharmaceutical or Industrial Intent [14][b].
	FDA CFSAN and CVM http://www.cfsan.fda.gov/	Additional oversight for food/feed safety	Federal Food Drug and Cosmetic Act (FFDCA)	See above [13]
	EPA http://www.epa.gov/	Reviewing APHIS Environmental Assessments and APHIS regulations;	Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). National Environmental Protection Act (NEPA) Toxic Substances Control Act (TSCA)	Not available

Table 2 (*Continued*)

Country	Authority	Scope of regulation	Laws and regulations	Specific regulations and guidance for pharmaceutical crops
	CFIA Plant Biosafety Office (PBO) http://www.inspection.gc.ca/english/plaveg/bio/pbobbve.shtml	Environmental release	Canadian Food Inspection Agency Seeds Act and Seeds Regulations	Directive 2007 (Conducting Confined Research Field Trials of Plants with Novel Traits in Canada) and its interim amendment for plant molecular farming field [9] Assessment Criteria for the Evaluation of Environmental Safety of Plants with Novel Traits Intended for Commercial Plant Molecular Farming [92] The PBO is currently developing a regulatory framework for the environmental release of plants which would require closed-loop confinement for commercial production due to potential food/feed, or environmental safety issues, a release termed commercial confined environmental release (CCER). The environmental release of plants intended for plant molecular farming is expected to be regulated under this new framework.
	CFIA Feed Section	Use of by-products as feed	Feeds Act and Feeds Regulations	n.i.
	CFIA Seed Section	Sale, advertising, import into and export from Canada of seed of pharmaceutical crops	Seeds Act and Seeds Regulations	Although there is no specific guidance pertaining to pharmaceutical crops, for most agricultural crops in Canada, variety registration is required before sale (Seeds Regulations, Part III)
	HC	Additional oversight for food safety		As part of the PBO-CFIA's regulatory framework for CCERs, proponents might be required to submit exposure and hazard data so that impacts on human and animal health resulting from exposure to the plant under review can be assessed. In addition, the potential hazards resulting from the unintentional introduction of plant material into the food and livestock feed chains will be assessed. It is anticipated that HC will review this exposure and the hazard data on behalf of the PBO.
	Member States National Competent Authorities	Field trials (Part B)	Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms [61][d]	Existing guidance is currently being reviewed to assess applicability to non-food crop usage. Specific guidance in preparation (announced by EFSA for 2008).
	European Commission EFSA Member States National Competent Authorities	Import, cultivation, processing, marketing for commercial purposes (Part C)		
		Applies to PMPs if (remainders) would be used as/in food/feed; might also apply if a food/feed crop would be used even if used for non-food/feed purposes only.	Regulation (EC) 1829/2003 on genetically modified food/feed [62]	
	European Commission Member States National Competent Authorities	Unintentional movements of GMOs between Member States and exports of GMOs to third countries	Regulation (EC) 1946/2003 on transboundary movements of genetically modified organisms [65]	Not available

Source: Adapted from references [93–95], http://www.inspection.gc.ca/english/plaveg/bio/mf/fracad/lovesure.shtml, http://www.bio.org/healthcare/pmpfactsheet4.asp.
Abbreviations: APHIS, Animal and Plant Health Inspection Service; CFIA, Canadian Food Inspection Agency; CFSAN, Center for Food Safety and Applied Nutrition; CVM, Center for Veterinary Medicines; EFSA, European Food Safety Authority; EPA, Environmental Protection Agency; n.i., not investigated; USDA, United States Department of Agriculture.
[a]Does not cover PMPs used for industrial purposes (e.g. the SemBioSys carp growth hormone).
[b]Does not cover PMPs used for food/feed purposes (e.g. the SemBioSys carp growth hormone).
[c]If manufacture, processing, distribution, use and/or disposal of a PMP produces a chemical substance that represents a risk to health or the environment.
[d]Transposed into national law of each Member State, and thus slight differences in the legislation might occur, especially for contained use and Part B field trials.

monitors GM plant imports, interstate movement (e.g. environmental safety issues and site inspections), the use of by-products, and the disposal of by-products and waste. The FDA also provides additional oversight to ensure safety of the food/feed chain.

In response to the concerns from the food industry and civil society organizations that PMPs might contaminate the food/feed chain, the USDA removed the notification track option, which is a simplified and fast-track procedure designed for agricultural GM crops intended for

food/feed. Furthermore, the USDA increased the criteria required for permission to cultivate PMP crops [45,50]. Draft guidance on the information required by applicants has been provided [13,14] but not yet finalized. However, these guidance documents are non-binding. According to APHIS, the planting of PMP crops requires continuous regulatory oversight, such that the producer must apply for a new permit every year and will not be eligible for deregulation (i.e. effectively releasing GM crops from regulatory oversight), which is currently the case for commodity GM crops following commercialization. More stringent confinement measures than those applied to conventional GM crops must be implemented. Such measures include increased isolation distances, fallow zones, increased inspection, and oversight (Box 3). In cases that require an Environmental Assessment (EA), which is associated with all environmental release permits, the applicant must provide additional information, including details of the potential for gene transfer to and persistence of the transgene in the environment, and the impact on plant and animal communities, agricultural practices and human health [14]. If human health is considered as being potentially affected, an EA also allows for a period of public comment.

The APHIS Compliance and Inspection Branch (CIB) was established in response to several violations of permit conditions [51]. US industry also launched a stewardship policy to 'enhance regulatory compliance and produce quality for consumers', which was recently broadened to become the 'Excellence Through Stewardship Initiative'. The latter initiative brings together the various stewardship measures on confinement of PMP crops, field trial compliance and insect resistance management, and introduces a third-party audit [52–57]. To accommodate the continuing criticism and litigation resulting from APHIS enforcement [58,59], the USDA established a Biotechnology Quality Management System (BQMS) in 2007, to complement the existing APHIS regulatory compliance and inspection process and to address compliance issues proactively together with applicants [60]. Both the Biotechnology Industry Organization (BIO) initiative and the BQMS aim to regain public trust and to prevent further trade disruption from non-compliance and adventitious presence.

Europe

Cultivation of all GM plants in the field constitutes an 'Environmental Release' and as such would require prior notification under Directive 2001/18/EC [61] to the National Competent Authority in the Member States. This Directive covers the deliberate release of food and non-food GM crops into the environment for both R&D purposes (Part B of the Directive) and commercial purposes (Part C), and it thus also covers any PMP crops grown in the field. To date, PMP crops have only been grown under Part B permits, ruling out their commercialization. Pathways for commercialization have yet to be addressed by the European Commission (EC), and it is therefore not entirely clear if applications can only be submitted under Directive 2001/18/EC. In this case, a national Competent Authority (CA) would evaluate the applications, and other national CAs would be asked to comment, with the European Food Safety Authority (EFSA) conducting its own evaluation in case of disagreements only. Alternatively, it is possible that application could be submitted under the centralized procedure set out in Regulation 1829/2003 on GM food/feed. In this case, EFSA would evaluate the application, and national CAs could make comments. With regards to PMPs, only applications for the commercial release of PMP non-food or 'food' crops that are not intended for food/feed purposes are likely to be evaluated under Directive 2001/18/EC rather than Regulation 1829/2003 [62], although this has yet to be clarified (Table 2). EFSA is currently addressing whether any of the existing risk assessment concepts and guidance for food/feed crops can be extended to cover PMP crops, including non-food crops. In 2006, EFSA initiated a self-tasking exercise to address such questions, and their results are scheduled for publication as a draft guidance document in 2008 [http://www.efsa.europa.eu/EFSA/Event_Meeting/GMO_Minutes_37th_plenmeet,3.pdf].

By contrast, the cultivation of PMP crops grown in containment would be regulated by the 'Contained Use' Directive, as amended by Directive 98/81/EC [63,64]. These regulations, overseen at the national level, are far less stringent than Directive 2001/18/EC, because containment does not necessitate a fully fledged environmental risk assessment. The export of live plants, including seeds, would fall under Regulation 1946/2003 [65] on the transboundary movements of GMOs, which would be especially relevant if seeds from PMP crops were exported to other countries (e.g. for field trials or commercial production).

Canada

Currently, the Canadian Food Inspection Agency (CFIA) regulates PMPs in the same way as other plants with novel traits (PNTs), using regulations set out under Canada's Seeds Act and Seeds Regulations (Part V). Canada is also developing these current regulations to cover the environmental release of PNTs specifically intended for commercial plant molecular farming (PMF) (Table 2). The CFIA is developing an approach that focuses on plants that constitute a potential risk to food/feed and/or environmental safety under this new proposed framework [66]. This new framework is likely to enforce a closed-loop production system that aims to keep PMP crops segregated from food/feed chains and, where appropriate, to minimize their environmental exposure. Developers of PMPs would be required to submit environmental, food/feed safety data, as well as to develop a release management strategy (RMS) as part of their application for 'commercial confined environmental release' (CCER) authorization. The applicant's RMS would outline how the developer plans to ensure that these crops would remain segregated from the food/feed chains and how dispersal into the environment would be minimized.

Plants authorized under CCER would then be subject to ongoing regulatory oversight, which would include on-site inspections during seed production, planting, growing, harvest and any post-harvest restriction periods (i.e. ensuring commodity crops are not grown in these locations in rotation). Off-site audits could also be carried out to

examine the developer's records on planting, seeding, monitoring, harvesting, corrective actions (where appropriate), and disposal and storage.

Australia

In Australia, PMP crops are subject to the same regulatory control as commodity GM crops, which are overseen by the Gene Technology Regulator, and both PMP and GM crops are assessed for risks to human health and environmental safety on the same case-by-case basis. The Gene Technology Regulator also has the authority to issue a license that contains specific conditions for managing risks. PMP crops and their products can also be subject to regulation by the Therapeutic Goods Administration, Food Standards Australia New Zealand, the Australian Pesticides and Veterinary Medicines Authority, or the National Industrial Chemicals Notification and Assessment Scheme, depending on the trait, plant species and intended use [67].

South Africa

The regulatory framework governing GMOs in South Africa requires permits for import and export, development, production, use, release and distribution of such organisms within the country. Since the GMO Act came into effect in 1997 [68], thousands of permits have been granted for conventional GM crops as well as for GM-derived pharmaceuticals from non-plant sources, but none have been approved for PMPs, to date. Among the public and private laboratories in South Africa registered to work on GMOs, only two are directly involved in PMP research – the Council for Scientific and Industrial Research (CSIR) and the University of Cape Town.

South Africa's biosafety system has been criticized for its weaknesses in terms of liability, public participation and access to information [69,70], and concerns have been raised that it might not be able to cater adequately for PMP crops [71]. One of the major concerns, as with most countries, is the issue surrounding contamination of the food chain. Therefore, the African Centre for Biosafety recommended that PMPs should not be produced in food crops.

The Cartagena Protocol on Biosafety

The CPB was established in an attempt to harmonize biosafety issues globally. As part of its remit, the CPB regulates the exchange of information among its 103 signature states as a prerequisite for permission for transboundary movements of GMOs. However, the CBP requirements are not mandatory in the main countries (e.g. USA, Canada and Chile) presently growing PMP crops in open fields, because these have not signed up to or ratified the Protocol, and as such are therefore not parties to the CPB. By contrast, the EU and several countries with recent interest in the technology, such as South Africa, South Korea and Japan, are parties to the CPB. The main CPB mechanism, the Advanced Information Agreement (AIA) procedure, establishes requirements and standards for risk assessment and the mutual exchange of information in case of imports and exports of GMOs, and this procedure is likely to be applicable to PMP crops only if viable plants or seeds intended for commercial exploitation are cultivated in open fields. The import of seeds from PMP crops that originate from field trials or commercial scale production outside the EU and which are intended for processing and extraction would trigger less extensive documentation requirements than the AIA would. Transboundary movements of processed plant material from PMP crops would fall outside the scope of the CBD [46,72].

Whether and how the CBD requirements will be tailored for PMP crops remains to be decided by the Conference/Members of the Parties of the Protocol (COP/MOP). A panel of risk assessment experts from academia, regulatory bodies and stakeholder groups gathered to advise the COP/MOP4 in 2008 and agreed that the general principles and methodologies for risk assessment laid out in the Annex of the CBP should be applied to PMP crops. Based on the experiences with PMP crops in some countries, the panel also identified extra requirements and knowledge gaps in risk assessment (e.g. on the pleiotropic effects of high-level expression, the environmental impact of PMP crop disposal, and occupational hazards [73]).

Regulations governing the licensing of pharmaceuticals derived from plants

Regulatory activities were triggered by PMPs entering clinical development, primarily within the FDA but also within its EU equivalent, the European Medicines Agency (EMEA). Although both authorities point out that the principles of guidance documents for other biopharmaceuticals apply, specific guidance has already been drafted in both jurisdictions to accommodate unique characteristics associated with PMPs. The respective policies of the different jurisdictions on orphan drugs and biosimilars (known as follow-on biologics in North America) also have a role, because some developers have such products (e.g. insulin, glucocerebrosidase) in their pipelines. However, these cases lie beyond the scope of this review.

USA

In the USA, the FDA oversees the licensing of most drugs and diagnostics, whereas veterinary vaccines are separately regulated by the USDA Center for Veterinary Biologics (CVB) (Table 3). In draft guidelines jointly developed by the USDA and the FDA, specific information is required for the market authorization of PMPs [13]. The FDA guidelines cover PMPs from all conceivable expression platforms, including transient expression using plant viruses, and stable expression in aquatic plants, moss and algae. These guidelines are therefore broader in scope than the corresponding draft from EMEA, which only covers stably transformed higher plants. When applied to PMPs, good manufacturing practice (GMP) guidelines appear to be more flexible at the FDA than at the EMEA (K. Webber [FDA], personal communication). With regards to plant characterization, the manufacturing process and pre-clinical testing, the information required for the commercial regulation of PMPs is similar – regardless of the chosen expression platform.

Europe

Pharmaceutical products derived from GM plants must adhere to the same regulation that covers all biotechnolo-

Table 3. Statutory authorities, regulations and guidance relevant for clinical trials and market authorisation of PMP products in the US, Canada and Europe[a]

Country	Authority	Scope of regulation	Laws and regulations	Specific regulations and guidance for pharmaceutical crops
	FDA www.fda.gov CDER, CBER, CVM	Biopharmaceuticals and vaccines for human use; biopharmaceuticals for veterinary use	Public Health Service Act (PHSA) Federal Food Drug and Cosmetic Act (FFDCA)	Draft Guidance for Industry: Drugs, Biologics, and Medical Devices derived from Bioengineered Plants for Use in Humans and Animals [13]
		Environmental effects from end products	National Environmental Policy Act (NEPA)	
	USDA-APHIS www.aphis.usda.gov CVB	Vaccines for veterinary use	Virus, Serum, and Toxins Act	
	HC www.hc-sc.gc.ca Health Products and Food Branch	Biopharmaceuticals and vaccines for human use	Food and Drugs Act and Regulations	No specific guidance yet; drugs derived from pharmaceutical plants are subject to the same oversight as normal drugs.
	HC Environment Canada	Environmental and indirect human health effects of new substances – either organisms or chemicals and polymers derived from organisms – before import into or manufacture in Canada that are not covered by other regulations scheduled under the CEPA	Canadian Environmental Protection Act, 1999 (CEPA). New Substance Notification Regulations (Chemicals and Polymers). New Substance Notification Regulations (Organisms)	Although there is no specific guidance pertaining to products derived from pharmaceutical crops, many of these products can be subject to the following guidance documents: Guidelines for the Notification and Testing of New Substances: Chemicals and Polymers [96]; Organisms [97].
	CFIA www.inspection.gc.ca Veterinary Biologics Section	Biopharmaceuticals and vaccines for veterinary use	Health of Animals Act and Regulations	No specific guidance yet; veterinary biologics derived from pharmaceutical plants are subject to the same oversight as normal veterinary biologics.
	EMEA www.emea.europa.eu CHMP, CVMP	Biopharmaceuticals and vaccines for human and veterinary use (assessment only)	Council Regulation (EEC) 2309/93 [74]	Guideline on the quality of biological active substances produced by stable transgene expression in higher plants [49].
	European Commission, National Competent Authorities	Market authorisation		

Source: Adapted from references [13,86,94,95,98].
[a]Regulations on medical devices are not included in this table. The plant-made antibody CaroRxTM is authorised in the EU as a medical device.Abbreviations: APHIS, Animal and Plant Health Inspection Service; BREC, Biologic and Radiopharmaceuticals Evaluation Centre; CBER, Center for Biologics Evaluation and Research; CDER, Center for Drug Evaluation and Research; CFIA, Canadian Food Inspection Agency; CHMP, Committee for Medicinal Products for Human Use; CVB, Center for Veterinary Biologics; CVM, Center for Veterinary Medicines; CVMP, Committee for Medicinal Products for Veterinary Use; EMEA, European Medicines Agency.

gically derived drugs, Regulation 2309/93 (Table 3) [74]. The relevant national authorities oversee these drugs during their research and early clinical development phases, and EMEA oversees them during commercial development and application. In 2002, EMEA published draft guidance notes on 'the quality of biological active substances produced by stable transgene expression in higher plants' [48], accompanying the similar document produced by the FDA (see above). Although the FDA guidelines have yet to be finalized, the EMEA guidelines were revised in 2006 and are still under development [49].

In 2004, a five-year EU-funded research programme called Pharma-Planta was launched. This programme had the specific aim to develop efficient and safe strategies for the production of clinical-grade PMPs and to work with the regulators to define appropriate guidelines [75–77]. Throughout 2007 and 2008, Pharma-Planta has been road-testing these latest guidelines by applying them to their own products and trying to help facilitate a better understanding of the specific characteristics of PMPs among regulators. The publication of successful case studies should reduce regulatory uncertainty, encouraging the industry to push their products towards the market; however, as stated above, several regulatory concepts originally developed for cell lines still need to be modified and redefined to be more specific for plants, especially those concepts surrounding master and working cell banks, compliance with GMP and, particularly with regards to batch-to-batch consistency, standard operating procedures for different production systems and downstream processing requirements. There is no 'natural' home or regulatory body for the entire start-to-finish responsibility surrounding the regulation of PMP crops and their products. As such, there is currently an overlap between authorities and a duplication of the information required by the different regulatory bodies, namely EFSA

and EMEA [78]. The precise stage at which each regulatory authority becomes involved, and the ways to deal with potential overlaps in their authority, is currently being investigated.

Canada

Health Canada (HC) is the federal authority that regulates the licensing of drugs in Canada. Before receiving market authorization, a manufacturer must present substantive scientific evidence about the safety, efficacy and quality of the product. The department is currently examining how these regulations apply to PMPs, and a common strategy still needs to be developed (S. Roussel [HC], personal communication).

WHO

The Third Global Vaccine Research Forum of the World Health Organization (WHO) mentioned plant-made vaccines (PMVs) as a potentially important issue [79]. In 2005, a 'WHO Informal Consultation on the scientific basis for regulatory evaluation of candidate human vaccines from plants' reiterated that existing guidance for the development, evaluation and use of conventional vaccines should be applied to PMVs. Other WHO guidelines on Good Agricultural and Collection Practices (GACP) for medicinal plants and for quality aspects of biopharmaceuticals can also be used for harvesting and for developmental genetics, respectively [80,81]. Specific issues that were flagged as being important include seed banking, dose control – in the case of orally delivered vaccines – and the risk of allergenicity. The existing principles of GMP for drugs and/or biologics were generally considered to be applicable, but would need to be modified and supplemented (e.g. by including GMP for early parts of manufacturing, such as agricultural and collection activities). Process validation under GMP was considered to be especially difficult in the case of open-field cultivation, and the Consultation therefore recommended greenhouse cultivation [82].

Outlook

The current PMP pipeline shown in Table 1 indicates that products from contained systems are on a faster track towards commercialization than PMPs from open-field sites. This partly reflects the perception that contained PMP production attracts a lower regulatory burden, but it might also in some cases reflect the choice of product. For example, high-margin, low-volume products will benefit from contained production, but there will be greater pressure for open-field production in the case of PMPs that give rise to lower-margin, high-volume products, such as nutraceuticals. For this reason, the pressure on regulators to develop a policy framework and appropriate regulatory pathways for the agricultural production of PMPs remains. However, given the concerns of the food industry, farmer groups and civil society groups, and the characteristics of the regulatory challenges, it seems likely that progress towards the regulation of open-field production, at least in the case of food/feed crops, will continue to be slow.

In the USA, the proposed revisions of the APHIS regulations – laid out in their Environmental Impact Statement (EIS) [83] – are likely to pave the way towards a more efficient framework for the cultivation of PMP crops. Instead of the currently applied zero-tolerance policy towards all PMP and PMI crops, a case-specific and risk-based policy formed around adventitious presence is envisaged. APHIS foresees a multi-tiered permit system that would differentiate between PMP and PMI crops, depending on the associated potential health and environmental risks and familiarity (i.e. knowledge of and experience with the crop), as opposed to the present situation in which all cases are considered to be equivalent. The degree of confinement and oversight would also be risk-proportionate and would vary per tier. An additional regulatory track would allow for the commercial production of PMPs and PMIs in open fields while still maintaining regulatory oversight. Multi-year permits are envisaged, although APHIS permit applications would be reviewed every year, even when locations and protocols have not changed [83]. A multi-tiered system is also supported by recent risk assessment case studies on PMP crop risk [25,26].

In Canada, work has been undertaken within individual departments of HC and the CFIA and also in a broader, inter-departmental working group that further includes Agriculture and Agrifood Canada, Canadian Grain Commission, Environment Canada and Industry Canada. This work aims to more clearly define the role that each department should have in the life cycle stages of plant molecular farming. It also aims to decide on the approaches that will be used to further develop regulatory frameworks. The specific strategy of HC is to develop an internal 'roadmap'. This will enable the involved parties to define regulatory pathways that different PMPs could take. By contrast, the CFIA is developing a regulatory framework for the environmental release of plants. This framework would require closed-loop confinement for commercial production, owing to potential food/feed or environmental safety issues.

In Europe, given the institutional separation of scientific risk assessment (undertaken by EFSA) and risk management (carried out at the EC level), the EC will be in charge of exploring and adopting its biotechnology framework. Some consider that confinement measures are part of risk management; as such, the scope of EFSA, which is normally limited to risk assessment, might need to be reconsidered. EFSA's Panel on Genetically Modified Organisms (GMO Panel) has launched an open consultation on the draft Opinion concerning 'the risk assessment of genetically modified plants used for non-food or non-feed purposes' (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178716609288.htm). These guidelines have the potential to intensify the debate at the level of the EC and within Member States. This might also stimulate discussions to determine how the present regulatory framework for GM crops could be adapted for PMP crops. Currently, the regulations only consider either small-scale non-commercial releases with regulatory oversight or larger-scale releases for unlimited commercial cultivation, processing and trade. The latter category, once approved, is excluded from regulatory oversight. With PMP crops, however, one can expect very small acreages, the absence of free trade in seeds and plant material, contract farming and strict confinement measures. Moreover, not only con-

cerned stakeholders but also the PMP industry will want to keep these crops under strict regulatory oversight [16]. In Europe, it might be difficult to reach agreement across all the Member States, because there is still a divergence of views, even with regards to risk assessment and risk management requirements for first-generation GM crops. Some Member States, including Austria, Hungary, Greece and recently France, are still pushing for stricter requirements. An appropriate regulatory pathway for PMP crops might therefore emerge only after a complex and lengthy negotiation process.

The present situation suggests that the overall approach to regulating PMP crops differs between jurisdictions, with the USA developing a tiered system, and Canada and the EU continuing their case-by-case approach. Regardless of the system, it is necessary to determine how the differences between PMP crops and GM food/feed crops will translate into risk assessment, confinement and monitoring requirements. Will extensive confinement measures and small plots result in less extensive risk assessment or is it anticipated that confinement failures justify fully fledged risk assessment and monitoring requirements [21]? EMEA already oversees pharmaceutical products derived from GM microbes and mammalian cells, and their draft guidance notes are continually revised to accommodate PMP-specific characteristics, such as defining master and working bank cells, cGMP compliance and batch-to-batch consistency. Although contained and controlled plant cell-based systems are likely to fit better into the current guidelines, other potentially important production platforms such as moss, *Lemna* and algae (not discussed in this article) are not yet included within the scope of EMEA's guidance, which focuses only on higher plants [49]. Additional regulations would be needed for alternative platforms such as transiently transformed plants [84] and GM plant viruses [85].

A general challenge facing emerging regulatory frameworks in the USA, Canada and the EU is the need to clarify the various and complex overlaps of regulatory oversight between different regulatory bodies, in particular between the USDA and the FDA in the USA, between CFIA and HC in Canada, and between EFSA and EMEA in the EU. A roadmap for applicants, clearly setting out the remits of these bodies and their responsibilities, could be helpful here.

Considering the issue on a more global level, industrialized countries are more likely to succeed in establishing a tight regulatory framework for PMP crops with rigorous confinement conditions that would be enforced by continuous oversight. Whether developing countries producing their own PMPs could establish and enforce such regulations remains questionable [73,86]. Countries that have weaker biosafety infrastructures could pose a risk to this emerging technology if 'contamination of the food chain' became an issue. This is of particular importance if developers conduct field trials and production in these countries. Strategies to avoid such problems will therefore have to be developed at the international level [87], especially in the contexts of the CPB, the Organisation for Economic Cooperation and Development (OECD), and the Codex Alimentarius.

The development of regulatory frameworks for commercial PMPs and the crops that produce them seems to be evolving by responding to real-world challenges rather than by anticipating them, because such frameworks are only slowly taking shape. Continuing regulatory uncertainty, by contrast, is discouraging PMP developers. To break this circle and to facilitate innovation in PMP development, regulators should adopt a more proactive stance. Nevertheless, a strong pipeline of PMP products would definitely facilitate regulatory development. Research and innovation policy might need to explore possible ways to support possible 'ice-breaker' products.

It is equally important that the regulatory frameworks are developed in an open and transparent manner, by including a broad range of stakeholders. This is particularly the case in Europe, and it might help to avoid or diminish the mutual suspicion and mistrust that has, for a long time, clouded discussions about first-generation GM crops.

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GUIDELINE ON THE QUALITY OF BIOLOGICAL ACTIVE SUBSTANCES PRODUCED BY STABLE TRANSGENE EXPRESSION IN HIGHER PLANTS

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GUIDELINE ON THE QUALITY OF BIOLOGICAL ACTIVE SUBSTANCES PRODUCED BY STABLE TRANSGENE EXPRESSION IN HIGHER PLANTS

TABLE OF CONTENTS

EXECUTIVE SUMMARY

Transgenic plant technology has emerged as a possible complement to the longer-established range of prokaryotic, yeast and mammalian cell-based recombinant protein production systems. In this document guidance is provided on the approaches which should be employed in order to achieve satisfactory quality for biological active substances proposed to be produced using the new technology.

1. INTRODUCTION

The principal aim of this guideline is to adapt aspects of the quality guidance already in place for other production systems to the special case of transgenic higher plant-based systems.

As is the case with other biotechnologically produced active substances, both the production process and its control play important roles in defining the quality profile of transgenic plant produced active substances. An additional consideration for transgenic plants-based production is that, since experience with the technology is limited, applicants are advised to be appropriately vigilant when conducting the development studies.

Methods used to stably transform transgenic plant genomes include micro-particle bombardment, micro-injection, *Agrobacterium sp.* mediation (for nuclear genome transformations), and *Chlamydomonas sp.* mediation (for chloroplast genome transformations). Typical distinguishing features of plants include growth on soil or aqueous substrates, the presence of tough cell walls, and protein processing patterns (including glycosylation patterns) which differ from those of other eukaryotic species. These features obviously have potential to impact on the quality, safety and efficacy profiles of the active substances produced.

2. SCOPE

The quality issues (including adventitious agent safety evaluation considerations) affecting biological active substances[1] produced by the expression of one or more transgenes stably located in the nuclear or plastid genomes of higher plants (meaning those belonging to the Spermatophytae (Gymnospermae and Angiospermae) taxonomic group constitute the scope of this guideline. Production using transiently transfected plants and production using plant cell culture fall outside the scope.

The guidance offered applies primarily to active substances intended for parenteral administration. For substances intended for non-parenteral administration, although all aspects of the guidance offered may not be applicable, applicants for Marketing Authorisation are reminded that the same general principles apply.

3. LEGAL BASIS AND CONSIDERATIONS

This guideline should be read in conjunction with the introduction and general principles (4) and part I, module 3 of the Annex I to Directive 2001/83/EC as amended, and with all relevant EMEA Committee on Human Medicinal Products (CHMP) guidelines. Aspects of certain EMEA Herbal Medicinal Products Committee (HMPC) guidelines, although addressing a different a usage of plant species, may also find applicability.

Medicinal products containing biological active substances manufactured using transgenic higher plants fall within the scope of the Annex to Regulation (EC) No 726/2004[2] and may normally only be

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[1] As defined in Directive 2001/83/EC, as amended. The biological substances produced in transgenic plants are typically recombinant proteins or peptides.

[2] Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency

placed on the market within the European Union if a marketing authorisation is granted in accordance with the Centralised Procedure as defined in this Regulation.

Containment/confinement measures applied to transgenic plant production systems are likely to function in the respective realms of medicinal product quality assurance (by protecting transgenic material from the environment) and environmental protection (by protecting the environment from transgenic plant material). Manufacturers responsible for cultivating or handling transgene-bearing plant tissue in the European Union need to comply with relevant Community Genetically Modified Organism and other environmental legislation, and in particular with Directive 2001/18/EC[3]. The measures in place should include those intended to prevent deliberate or accidental ingestion of transgenic plant parts by animals or human beings, either via direct consumption, or through inadvertent release into food or feed supply chains.

4. MAIN GUIDELINE TEXT

4.1 Development genetics

4.1.1 The host plant

Applicants should document the rationale for the choice of host plant for the genetic manipulation, taking into account attributes such as phenotype/genotype variation and stability, suitability for routine cultivation in manageable environments, susceptibility/resistance to infection with extraneous agents (for example, plant viruses/viroids, and fungi), and post-translational patterns for proteins.

The chosen host plant should be defined in terms of family name, genus, species, sub-species, cultivar/breeding line and common name, quoting the classifying authority. The host plant may itself be engineered to express specific traits and characteristics, such as modification of the plant glycosylation process, growth performance, or resistant features. In such cases, the development of the engineered host plant should be described in detail, and the chosen strategy should be explained.

Appropriate purification strategies should be developed, and a risk assessment should be presented, if the host plant is known to produce constituents potentially harmful to humans such as secondary metabolites (for example, pharmacologically-active alkaloids or glycosides).

4.1.2 The transgene and expression construct

The manufacturer should describe the origin of the nucleotide sequence coding for the protein. All subsequent modifications of the DNA sequence should be identified and described.

The method of transformation used to generate the initial transformant should be justified, and the assembly of the expression construct should be described in detail. When using micro-organism-mediated transformation, for example using *Agrobacterium sp.*, full documentation on the origin, history, and biological characteristics of the system should be provided. The description of the expression construct should include the source and function of the component parts, for example, origins of replication, selection marker or reporter genes, promoters, enhancers, and leader/targeting sequences. A detailed component map and a complete annotated sequence of the plasmid should be given, indicating those regions that have been sequenced during the construction and those taken from the literature. The nucleotide sequence of the coding region of the gene of interest and associated flanking regions that are inserted into the vector, up to and including the junctions of insertion, should be determined. Other expressed proteins encoded by the plasmid should be indicated. Genetic material other than the gene of interest that are introduced or altered to regulate or modify a specific trait of the host plant (for example, factors affecting expression or inhibition of glycosyltransferase, factors affecting dissemination) should be documented and explained.

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[3] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC

4.1.3 Generation of the primay transformant

The description of procedures and materials employed for the transformation event should be presented. The status of the genetic material incorporated, or modified, should be documented for the primary and/or final transformant, as appropriate. This documentation should include at least information on the desired sequences, number of loci and inserts, tandem repeat, inverted repeat, sequence of insert, flanking regions, junctions of insertions, residues of process materials remaining from the transformation process (for example, the fate of *Agrobacterium* infection).

4.1.4 Generation of the final transformants

Primary transformants produced by the transformation event are typically bred through a series of generations to produce final or production transformants. In the Marketing Authorisation Application these may be designated T0 (for primary transformant), T1, T2, T3 etc for successive generations, and Tp for the production transformant, or an alternative system of nomenclature may be used if the circumstances warrant it. The operations involved should be described in detail, including information on all manipulations, reagents and media used.

If an elite plant line is employed in the process, a justification should be provided, and complete details as for the main transgenic line should be provided. The crossing events should be described in detail, and the impact of the crossing event on the properties of the generated plant line determined and described.

4.1.5 Transgenic banking system

Where possible and unless otherwise justified, a banking system should be included in the batch-to-batch consistency assurance strategy. Depending on the production strategy, there may be a need to bank both the production strain and an elite line. The fundamental principles underlying banking systems for substrates and materials used in the production of biological medicinal products are outlined in CHMP guidelines, and should be taken into account by manufacturers of transgenic plant-derived active substances when designing their systems.

Manufacturers should therefore establish a master and working transgenic bank of plant material derived from the final transformant, capable of long-term storage and of providing consistent and sufficient starting material for a number of production runs which is sufficiently large to ensure long-term continuation of supply.

The generation, establishment and maintenance of both the master and the working transgenic banks should be defined and clearly described. The approach applied to characterising and testing the master transgenic bank and the working transgenic bank should take into account the guidance outlined in CHMP guidelines, with adaptation to the particular transgenic plant production system in question. The plant material used to establish the master transgenic bank should be thoroughly characterised genotypically and phenotypically. The characterisation of the material used to form the master transgenic bank should include a comparison of its botanical, horticultural, agricultural and phytochemical characteristics with its natural counterpart, with a view to identifying any emerging characteristics which might have significance for the production crop, such as gene silencing activity or pleiotropic effects resulting from the presence of the transgene, which might have consequences for the quality, and safety of the active substance.

This study should include an analysis of the transgene (for example, sequence(s), integrity, site(s) of insertion, copy number, and fates of marker sequences), its expression (tissue/organ specific, regulation, and expression level), plant gene silencing effects, over-expression of other proteins, ploidy, and karyology).

The stability behaviour of the banked material should be investigated and on the basis of the results the following should be defined:

- Specifications for container and closure systems.
- Storage conditions.

- Shelf-life.

4.1.6 Genetic stability

The genetic stability should be determined for the production system, from the primary transformant stage through to the crop at time of harvest. Data from successive crops should be included in the determination. A limit of plant age for the intended culture conditions should be defined. Genetic stability studies should be complemented with supportive data obtained from in-process controls during cultivation, and the results of control testing of the batches of the active substance. It is important to inter-relate these issues in Marketing Authorisation Applications.

4.2 Manufacturing issues

4.2.1 General manufacturing strategy

For all biological active substances, the production system and its control is one of the factors determining both the consistency of production and the quality of the material produced. In the case of production of active substances using transgenic higher plant technology, a clear strategy for implementing this principle should be proposed, and illustrated by means of a flow diagram.

Good production practice

The Master Transgenic Bank and the Working Transgenic Bank should normally be established and maintained under GMP conditions.

The production process of each batch of active substance should be considered to start with an aliquot taken from the working transgenic bank and to conclude with the testing and release of the batch.

Production processes employing transgenic plants can normally be divided into two distinct phases.

The first production phase is specific to transgenic plant technology and includes the cultivation, harvest and primary processing (for example screening, cleaning, sorting, macerating, transporting and/or storing) of the harvested material. Where classical GMP principles prove impractical to apply to elements of this phase, a suitable Quality System should be developed and put in place.

The heading used to describe the Quality System should include at least personnel, including qualifications and training, documentation including traceability, arrangements for audits and inspections, and information on whether the system is certificated by any official organisation. The development of the System may use as a starting point the basic principles outlined in the HMPC "Guideline on Good Agricultural and Collection Practice (GACP) for Starting Materials of Herbal Origin", though confirmation of compliance with the GACP Guideline, which is aimed at a different usage of plants, is not alone considered adequate for controlling transgenic plant-based production.

Ultimately, whether performed in accordance with GMP or with a defined quality system, the early steps of the manufacturing process should be well controlled by the application of suitable in-process controls, provide a well-defined starting material suitable for subsequent processing under GMP, and be well documented. The operations and the documentation should be available for inspection.

Production operations for the active substance downstream of primary processing (the second production phase) should normally be conducted according to GMP. The second phase, encompassing product isolation, purification, formulation, etc., is common to all biotechnology-derived products and the general requirements are documented in the relevant CHMP and GMP guidelines.

4.2.2 First production phase

Description of the site

- Geographical location, with boundaries exactly defined.

- The quality and nature of the growth substrate (typically soil, aqueous solution, or aqueous suspension), water supply and other raw materials (including fertilisers and pesticides) should be defined, and specifications should be set, where appropriate.
- The prevailing meteorological conditions, with seasonality and general variability should be documented. Extreme conditions for the locality should also be mentioned.
- Supervision of the site.
- Local flora and fauna.
- Cultivation of other genetically modified plants in the vicinity.
- The quality and/or good practice system in operation at the site.

Procedures for cultivation

- Propagation steps and techniques. Depending on the cultivation strategy, the number of generations should be clearly defined for each step with reference to the documented genetic stability of the process.
- Procedures for the detection and removal of undesirable plants and ingress of foreign genetic material, including pollen.
- Procedures for the detection and removal of pests.
- Procedures for monitoring the status of plant health, plus actions to be taken in case of disease.
- In-process monitoring of production consistency. The critical parameters for cultivation should be defined and justified, and are likely to include:
- Planting technique and location, taking into account environmental conditions including seasonality and nature of neighbouring flora.
- The nature of the soil substrate (including potential radioactivity).
- Plant hormone and fertiliser application.
- Pesticide application, including the use of chemical and biological agents.
- Potential for genotype proliferation arising from sexual reproductive techniques.

Harvesting and primary processing

- Criteria for initiation of harvesting.
- Harvesting technique including techniques to prevent contamination with rodents, birds and carcasses.
- Procedures and validation of the immediate manipulation of biomass once harvested, including transport and storage arrangements, and mechanical, physical, chemical and biological treatments applied.
- Conditions and duration of storage of isolated primary-processed material

The definition of a batch of post-harvesting material, active substance and final product should be provided, and the arrangements for the traceability of each batch back to the original unit of the Working Transgenic Bank should be described. Provisions for pooling of harvest or any other intermediate should be defined, and where appropriate, specifications should be set.

4.2.3 Second production phase (downstream processing)

As is the case with biotechnology-derived medicinal products generally, the methods used to purify the product and their in-process controls including their specifications should be described in detail, justified and validated. Considering the specificities inherent to plant cultivation, particular attention should be placed on the demonstration of the robustness of the production processes.

Potential impurities or contaminants derived from the plant and the production process (for example, host-cell proteins, DNA, plant metabolites, herbicide, fertiliser, and mycotoxins) should be evaluated. Care should be taken to document host proteins homologous to the required product, contaminants which may co-purify with the desired material, and any elements with potential to raise safety concerns (including hypersensitivity reactions).