Chapter 17

Regulation of Molecular Farming Products

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Abstract

The regulation of molecular farming is a complex topic because plants and plant-based systems are relative newcomers among the many production platforms available for recombinant proteins. The regulations specific for different types of product (human/veterinary pharmaceuticals and medical devices, cosmetics, diagnostics, and research reagents) must therefore be overlaid with the regulations governing hitherto unfamiliar production platforms, and this must be achieved in different jurisdictions that handle genetically modified organisms (and genetically modified plants in particular) in very different ways. This chapter uses examples of different product types and production methods in three different jurisdictions (the USA, the EU, and Canada) to demonstrate some of the challenges facing the regulatory authorities.

Key words Cosmetic, Diagnostic, GMO, Good manufacturing practice, Medical device, Pharmaceutical, Plant cells, Research reagent, Transgenic plants, Transient expression

1 Introduction to Molecular Farming

Molecular farming is the use of plants and other plant-derived systems (such as cell suspension cultures, hairy roots, and other tissue explants) for the manufacture of recombinant proteins (*see* **Note 1**). Molecular farming in whole plants was developed as a concept in the late 1980s and was initially promoted on the basis of three key advantages over traditional fermenter-based systems: scalability, safety, and low costs [1–4]. More recently, with the advent of transient expression systems, the combination of speed and scalability has emerged as an additional advantage, particularly in the context of pharmaceutical products targeting rapidly spreading infectious diseases, including most recently COVID-19 [5]. Plants have also been at the forefront of the development of glycan engineering technology, allowing for the production of pharmaceutical proteins with humanized or otherwise modified glycans [6, 7]. From a regulatory perspective, the classification of

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molecular farming is important because it defines the product as the recombinant protein whereas the plant is merely the vessel in which the product is manufactured, thus contrasting with other forms of agricultural technology in which the modified plant is the product—for example, pest-resistant crops. The recombinant protein is either purified from the plant tissue or is used as part of the plant tissue matrix, such as an orally administered vaccine expressed in edible tissues such as seeds and fruits [8]. Even so, the regulatory systems governing molecular farming apply to both the production system and the product, with complex and often conflicting results [9].

The regulatory complexity arises from several sources, which can be summarized as follows.

- Strictly regulated biologic products are generally produced in clonal platforms such as mammalian cell lines, bacteria, or yeast, which satisfy the requirements of good manufacturing practice (GMP) in terms of genetic uniformity and product consistency, whereas nonclonal systems such as whole plants largely fall outside this definition (*see* **Note 2**).
- The most strictly regulated processes are those involving the release of genetically modified organisms (GMOs) or living modified organisms (LMOs) into the environment (*see* **Note 3**).
- Different regulatory systems are in operation in different national and regional jurisdictions and the degree of harmonization is greater for purified products but much less so for crude product formulations and GMO/LMO production platforms.

Initially, the general outcome of these three overlapping factors was that molecular farming in whole plants, especially in the open field, was considered too different from established practices to convince pharmaceutical companies to consider plants as a feasible alternative to fermenters (despite the advantages) and that the higher regulatory burden (and corresponding increased costs) attracted too much additional perceived risk [10]. The molecular farming community has addressed this challenge in two ways—first by developing systems that fit more closely with regulatory standards for other platforms (heterotrophic plant cell suspension cultures, autotrophic single-celled organisms such as algae, and autotrophic clonal plants such as moss and duckweed) and second by engaging with regulators to develop amendments to existing regulations or new regulatory standards that accommodate sexually reproducing transgenic plants and transient expression systems based on bacterial infiltration and/or plant viruses [11–13]. There is still some way to go to catch up with established platforms such as CHO cells, where there is regulatory consistency based on well-defined processes, best practices, and standards. However, the molecular farming community working with whole

plants has begun to consolidate around a small number of platforms (particularly transient expression in *Nicotiana benthamiana* and the stable transformation of tobacco and cereal crops and their cell lines) which could lead to more regulatory harmony in the future [4].

In this chapter, we summarize the regulatory processes for the approval of different types of products in three different molecular farming platforms in three jurisdictions to give a flavor of the challenges facing the molecular farming community. In the product category we discuss (1) pharmaceuticals, which are subject to the strictest regulation, (2) other regulated products such as medical devices, veterinary products, and cosmetics, which are subject to a lower regulatory burden than pharmaceuticals, and finally (3) diagnostic reagents and technical enzymes, which are not yet fully regulated beyond standard consumer protection. It is important to recognize that the intrinsic nature of the product is less important than its application—for example, the same antibody or growth factor can be developed as a pharmaceutical, medical device, cosmetic ingredient, or research-grade reagent but the process would be regulated differently in each case (*see* **Note 4**). In the process category we consider (1) transgenic plants (strongest regulation, particularly for deliberate environmental release), (2) transient expression, and (3) clonal systems such as cell suspension cultures. One of the important take home messages is that the regulations affecting these processes mainly differentiate between the upstream production phases, which are the most diverse. By the time the product has been extracted into a sterile buffer for downstream processing (DSP), there is little if any difference between the regulation of a molecular farming product and an equivalent product extracted from CHO cells or bacteria. This is because DSP is more focused on the characteristics of the product than how it is produced. In the jurisdiction category, we consider molecular farming in the EU, the USA, and Canada. There are significant differences in the approaches each jurisdiction takes to the regulation of molecular farming, although product-level regulation for pharmaceuticals follows the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) to improve compatibility between different jurisdictions for the approval of pharmaceuticals [14].

2 Approval of Pharmaceutical Products

2.1 General Regulations for the Approval of Pharmaceutical Products

We consider pharmaceutical products first because they are the most strictly regulated products. These regulations are not specific to plants but need to be considered for plant-made pharmaceuticals. All jurisdictions follow a multiphase development path with four steps. First, preclinical development is conducted in accordance with ICH S6 guidelines, including pharmacology studies in

animal models, rodent and nonrodent toxicology studies, and safety studies. Second, clinical development involves Phase I safety and pharmacokinetics, and/or immunological studies; Phase II dose-ranging, safety, and efficacy studies; and Phase III pivotal safety and efficacy studies with defined primary and secondary endpoints. If clinical studies are successful, the third step involves filing a licensing application, a regulatory agency review to determine whether the benefit outweighs the risk for the indication, and approval for marketing. Finally, the development path involves postmarketing commitments and requirements. The similarities and differences between jurisdictions have been widely discussed [15, 16] and are summarized below.

1. In the USA, the transition from preclinical to clinical development requires an investigational new drug (IND) application to the Food and Drug Administration (FDA). This is generally preceded by requesting a pre-IND (PIND) meeting with the appropriate FDA review division to discuss the development plan including preclinical studies; chemistry, manufacturing, and control (CMC); and a Phase I design and starting dose to ensure the IND is acceptable. Although such meetings are not mandatory, they are strongly recommended. There is also an opportunity to discuss the nonclinical development plan (including toxicology) before the pre-IND stage.
2. In the EU, the transition from preclinical to clinical development requires a clinical trial application (CTA) to a national regulatory agency in the member state where the clinical trial will be conducted. An investigational medicinal product dossier (IMPD) which includes nonclinical and CMC information is submitted beforehand to provide necessary information for the review of the proposed protocol as part of the CTA.
3. In Canada, the transition from preclinical to clinical development also involves a CTA, which contains quality information pertaining to the investigational drug, copies of the clinical trial protocol, a model informed consent form, and the investigator brochure. The latter includes nonclinical information, clinical information (if available) and a statement of the risk and benefits to the health of participating trial subjects. CTAs must also contain an attestation signed by a senior medical or scientific officer in Canada and the senior executive officer containing information dealing with the drug, its destination, or the clinical trial. Clinical trial sponsors may submit an application to request a pre-CTA consultation meeting with Health Canada as an opportunity for the sponsor to present relevant data, discuss concerns, and receive guidance on the acceptability of the proposed trial.

Clinical development involves a Phase I trial to collect safety and pharmacodynamics and/or pharmacokinetics data. If safety is acceptable, the clinical candidate moves to Phase II trials to establish dose and preliminary efficacy of the drug, usually in comparison to a placebo control with small test groups. Phase II trials may be divided into IIa (dose-ranging) and IIb (therapeutic dose range) testing. Phase I/II trials may be conducted at the same time for some products, such as preventive vaccines. Phase III trials are well-controlled trials designed to confirm safety and efficacy in a larger population and are required to enable the application for licensing or approval and market authorization. Further postapproval studies of efficacy and safety are described as Phase IV, but are not always required. Sponsors are organizations with overall legal responsibility for a clinical trial, and must ensure that it complies with the relevant legislation. Sponsors are encouraged to meet with the regulatory agencies between clinical phases, most importantly the end of Phase II to discuss the design of the Phase III trials and the regulatory pathway.

4. In the USA, the successful completion of Phase III trials is followed by a new drug application (NDA) for synthetic drugs or a biologics license application (BLA) for vaccines, blood products, cell and gene therapy products, and proteins/peptides, including molecular farming products (*see* **Note 5**).
5. In the EU, the successful completion of Phase III trials is followed by a marketing authorization application which can be submitted to the competent authority in any member state or to the European Medicines Agency's (EMA) Committee for Medicinal Products for Human Use (CHMP). The latter process is mandatory for biotechnology-derived medicines including molecular farming products.
6. In Canada, the launch of a new pharmaceutical drug requires the filing of a new drug submission (NDS) with Health Canada's Biologic and Radiopharmaceutical Drugs Directorate (BRDD). This incorporates the preclinical and clinical testing, data about the production of the drug substance and the drug product, and also information on packaging, labeling, stability, and validation procedures.

   Multiple molecular farming products have reached Phase III development but most have taken advantage of accelerated approval pathways (as described in the next section). The first molecular farming product to complete Phase III trials through the standard clinical development route was Medicago's quadrivalent influenza vaccine based on virus-like particles (VLPs) consisting of hemagglutinin proteins from four virus strains. This vaccine candidate completed Phase III development in 2020 [17, 18].

2.2 Accelerated Approval Routes for Pharmaceuticals

All jurisdictions have special measures for the approval of particular pharmaceutical products based on the type of disease indication, including emergency scenarios such as outbreaks of epidemic or pandemic diseases [19]. It is important to note that all molecular farming products approved as pharmaceuticals until the end of 2020 followed one of these pathways.

1. Orphan drugs are developed to treat rare diseases (*see* **Note 6**) and benefit from special financial concessions in most jurisdictions because the small number of patients would not otherwise make such drugs profitable. In addition to the financial incentives, orphan drugs often benefit from an accelerated clinical development program because Phase III clinical trials may not be possible, or may only be possible with a smaller than normal cohort, because it is difficult to find enough patients for recruitment. The first molecular farming pharmaceutical product approved for human use was an orphan drug (taliglucerase alfa) developed by the Israel-based company Protalix Biotherapeutics for the treatment of Gaucher's disease [20].
2. Drugs that have not completed clinical development for a particular indication may be authorized on a temporary basis in an emergency if their use is judged to be beneficial, as seen for certain antiviral drugs in response to the 2009 swine flu pandemic and in 2020 for COVID-19. These temporary authorizations are known as emergency use authorizations in the USA, conditional marketing authorizations in the EU, and interim orders in Canada. They are not approvals per se, and additional clinical development may be required once the temporary authorization expires. Although no molecular farming products have yet been subject to emergency use authorizations, the ZMapp antibody cocktail purified from infiltrated *N. benthamiana* plants was approved under the animal efficacy rule, which allows use in humans based on animal data if clinical studies would be impossible or unethical [21]. This was deemed to be the case in the 2014–2016 outbreak of Ebola disease in West Africa, in which approval was given for seven patients to receive the ZMapp antibody cocktail based on data obtained in primates [22] and five survived [23]. As with the emergency use authorization, products approved under the animal efficacy rule are expected to continue with clinical development in parallel [24].
3. Special approval pathways have also been introduced for the development of drugs against COVID-19, including the Coronavirus Treatment Acceleration Program (CTAP) launched by the FDA in March 2020 [25] and the COVID-19 Pandemic Emergency Task Force [26] based on EMA's Emergent Health Threats Plan published in 2018. Neither of these pathways

changes the regulatory burden on new pharmaceutical products but they massively cut the developmental timelines, especially the CTAP which reduces typical response times from months to days or even hours.

2.3 Special Regulations Applicable to Molecular Farming Products

The transition from preclinical to clinical development is marked by a requirement in all jurisdictions to meet standards for the quality of the manufacturing process that affect the quality and consistency of the product, which is at this stage known as an active pharmaceutical ingredient (API). These standards are collectively known as GMP and cover multiple aspects of the production process, including the quality and consistency of raw materials. Most biologics are produced in bioreactors containing cells, so the environment is fully contained, and the conditions can be precisely controlled and monitored. Ideally, all raw materials are completely characterized including the culture medium (preferably a chemically defined medium) and the cell line. To ensure batch-to-batch consistency, the cell line is prepared as a frozen stock known as the master cell bank from which aliquots are withdrawn to seed working cell banks used for each production campaign. The master and working cell banks must be fully characterized to confirm cell identity and purity, and the stability of the genetic modification.

As stated above, the molecular farming community has addressed the GMP requirements on one hand by developing platforms based on plant cell suspension cultures, which are precisely analogous to mammalian cells and microbes, even offering some advantages (such as the inability to support the replication of mammalian viruses and the absence of endotoxins as produced in bacteria such as *Escherichia coli*). The first approved pharmaceutical product derived from plant-based molecular farming was produced in plant cell suspension cultures [20] and the FDA subsequently agreed that clonal plants grown in bioreactors (algae, moss and duckweed) were similar enough in character to be accommodated under the same regulations [27]. The special regulations needed for molecular farming therefore pertain mainly to the use of systems based on whole plants [28–30].

1. The USA regulates molecular farming in a conceptually similar manner to any other biotechnology-derived product and therefore focuses more on the product than the process. Accordingly, the products and reagents are covered by the U.S. Code of Federal Regulations (CFR) Title 21 (Food and Drugs) including current good manufacturing practice (cGMP) covered by 21 CFR Parts 210211, good laboratory practice (GLP) toxicology (21 CFR 58), and a collection of good clinical practice (CGP) requirements specified by the ICH and accepted by the FDA (especially ICH E6 R1, R2, and draft R3).

2. In the EU, biopharmaceuticals are regulated by the EMA, and also by the competent local authority of the individual member state. Pharmaceuticals from genetically modified plants must adhere to the same regulations as all other biotechnology-derived drugs. These guidelines are largely specified by the European Commission (EC) in Directive 2001/83/EC and Regulation (EC) No 726/2004. However, upstream production in plants must also comply with additional statutes relating to environmental release if grown outdoors (Directive 2001/18/EC, and 1829/2003/EC if the crop can be used as food/feed) and Directive 2009/41EC if grown in containment, such as in a greenhouse (*see* Subheading 4).
3. In Canada, biopharmaceuticals are regulated by Health Canada under Schedule D of the Food and Drugs Act. Biopharmaceuticals produced in plants are handled under the same regulations except those defined as natural health products or drugs produced in plants by conventional breeding or horticulture. Existing Health Canada and ICH guidelines for the manufacturing of active ingredients, drug substances, and drug products in cell culture are therefore applied to molecular farming. Special measures applying to molecular farming products have been set out in the guidance document Plant Molecular Farming (PMF) Applications: Plant-Derived Biologic Drugs for Human Use [30]. The biologic product itself is regulated by Health Canada's Health Products and Food Branch (HPFB) under the Food and Drugs Act. Upstream production in plants may be regulated by CFIA (Plant Biosafety Office) under the Seeds Act and Seeds Regulations or the Plant Protection Act and Plant Protection Regulations. The product may also fall under the oversight of the New Substances Program jointly administered by Health Canada and Environment and Climate Change Canada (ECCC).

2.4 Molecular Farming: Biological Raw Materials

One of the most important GMP concepts is the quality, consistency and traceability of raw materials, including the biological materials used to initiate upstream production. In conventional fermenter-based manufacturing this would be the cell line, a well-characterized clonal line maintained as a stock known as the master cell bank, which is used as a resource to prepare working cell banks for each production campaign. The master and working cell banks are important because they ensure that the quality and consistency of living cells does not change over time through multiple passages. Each working cell bank is guaranteed to be only a certain number of generations removed from the master cell bank, reducing the likelihood of mutations leading to subclonal variation.

In the context of molecular farming, plant cell suspension cultures and other clonal systems can largely adhere to the master/working bank concept, allowing the raw materials to be

maintained under GMP conditions. For whole plants, strict clonality is not possible in most species (due to sexual reproduction and the inherent variation this introduces) but GMP-like standards can be implemented by ensuring the adequate characterization of stock plants, such as the provision of seeds from an accredited source [4, 31]. The use of a master seed bank as a direct equivalent of the master cell bank allows the preparation of working seed banks for each production campaign by breeding a set number of generations from the master seed in order to obtain a sufficient number of plants. For transient expression systems, the master seed gives a production batch which is then infiltrated with bacteria maintained using the traditional GMP-compliant master/working cell banks. For transgenic plants, the transgenic stock must be characterized to ensure the locus carrying the transgene is intact from generation to generation.

2.5 Molecular Farming: Upstream Production

In all jurisdictions, it is important to note that issues surrounding the GMP compliance of whole plants have not been solved directly, but have been sidestepped by segregating the GMP and non-GMP parts of the process [4, 31]. Essentially, for both transient expression systems and transgenic plants, the plant takes on the role of a self-contained disposable bioreactor and is discarded before the GMP process begins (*see* **Note 7**). Cell suspension cultures and other clonal systems are grown in physical bioreactors under axenic conditions and are handled in the same manner as other fermenter-based production platforms. Whole plants cannot be grown in a sterile environment, so the expectations of the regulators are that GMP-like principles are implemented in terms of process monitoring and traceability.

1. The upstream phase includes all manufacturing operations involving plant or plant cell cultivation, infiltration (transient expression only), harvest, and in some cases the initial extraction steps. GMP production only begins when a sterile extract is available.
2. Although whole plants cannot achieve GMP standards, good agricultural and collection practices (GACP) are recommended to ensure product quality and consistency. Upstream process efficiency has been shown to vary considerably with even minor variations in factors such as temperature and light. Such variations in the upstream process can also affect the efficiency of downstream purification steps, potentially leading to changes in product quality. Steps taken to ensure the reproducibility of plant growth conditions (such as regulated temperature, humidity, and gas composition; LED lights for constant light quality; uniform substrates such as Rockwool instead of soil; optimal spacing to prevent crowding and shading; and feedback-optimized nutrient/fertilizer solution) can increase

batch consistency, analogous to the controlled conditions of traditional bioreactors (*see* **Note 8**).

3. Clearly defined manufacturing processes must be established to ensure consistency and compliance with approved specifications including the use of pesticides, soil, water, or fertilizer, and for the qualification of plant health, height, weight, or other developmental characteristics. Quality systems in the upstream process help to generate a defined biological starting material suitable for subsequent downstream processing under GMP conditions. Manufacturers are expected to justify the GMP-like principles applied during the upstream process.
4. Elements of process analytical technology can be incorporated into plant-based production processes to allow automatic feedback control of the greenhouse environment, analogous to the measurement of temperature, pH and dissolved oxygen in conventional bioreactors and automatic correction to keep within the critical process parameters (*see* **Note 9**).

2.6 Molecular Farming: Downstream Processing

Downstream processing (DSP) in conventional platforms (and those based on plant cell suspension cultures) begins when the product is separated from the production organism. The extract, without cells or tissues, is then subject to multiple fractionation steps to remove particulates and soluble impurities. In the simplest systems, cells secrete the product into the medium and downstream processing begins after a simple filtration step to remove the cells from the fermentation broth. Secreted proteins can be recovered from plant cell suspension cultures and other clonal systems (*see* **Note 10**). However, in the vast majority of whole plants the product is intracellular and must be extracted by a disruptive initial process such as shredding or grinding plant tissues to break open the cell walls [32] or by cutting the leaves into pieces and soaking them in an extraction buffer containing enzymes that depolymerize the cell wall [33].

1. The first DSP steps with whole plants take place under non-GMP conditions because the plant tissue is not sterile. Some tissues present a low contamination risk (e.g., transgenic rice seeds) but others are teeming with bacteria (e.g., transient expression involves the deliberate introduction of a diluted bacterial suspension into plant tissues). A typical first step is therefore to extract proteins from plant tissue by shredding/grinding or cell wall depolymerization in a buffered solution. In addition to the target protein, the crude extract contains plant fibers, bacteria and viruses, and particulates over a large size range in addition to soluble impurities. The crude extract is then processed by centrifugation and/or filtration to remove particulates and bacteria, resulting in a cleared extract that can be filtered into aseptic bags ready for the GMP facility.

2. The material entering the GMP process is the green juice or equivalent which has typically been prepared by homogenizing the plant tissue and passing the homogenate through a series of depth filters and a final dead-end filter to yield a clarified feed stream. All process operations from that point forward to the final drug substance are guided by 21 CFR 201/211 (cGMP) in the USA, and Directive 2001/83/EC and Regulation (EC) No 726/2004 in the EU.
3. Once the clarified feed has entered the GMP facility it is handled in exactly the same way as the fermenter broth from a conventional GMP facility and no additional regulatory burden applies. Some of the purification steps may be unique to plant-based material, including the removal of a larger quantity of host cell proteins (*see* **Note 11**) and specific metabolites (*see* **Note 12**). There may also be a need for endotoxin removal (*see* **Note 13**). Plants do not support the replication of human viruses but measures are still required for the reduction or removal of adventitious agents [13].
4. The unique properties of the production system should be accommodated into the quality information reported to the regulators. Such information should include plant identification and description, expression systems, generation of banking system, upstream preharvest/harvest production phase, downstream postharvest production phase, impurities and potential for allergenicity, manufacturing process development, release testing and specification limits, and control of endogenous and adventitious contaminating agents.

3 Other Regulated Products

3.1 Medical Devices

The definition of a medical device is very broad, covering not only what might be described as conventional devices (ranging from simple tools like tongue depressors and swabs to complex instruments such as pacemakers and prosthetics) but also molecules such as proteins. A protein can be a medical device as long as it does not "... achieve its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of its primary intended purposes ..." (FDA definition) or "... achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means ..." (EMA definition). This means proteins produced by molecular farming that meet this definition can be classed as medical devices and regulated as such, which attracts a lower regulatory burden than pharmaceuticals. An example of a molecular farming product approved as a

medical device is CaroRX, a monoclonal antibody that prevents the colonization of teeth by the bacterium *Streptococcus mutans*, thus helping to prevent dental caries [34].

1. In the USA, medical devices are regulated by the FDA under CFR Title 21 part 860 and are divided into three classes based on risk, with class I the lowest and class III the highest. Class I devices are not subject to regulation because they are deemed to pose no significant risk (e.g., bandages and surgical gloves). Class II devices have special labeling requirements and require postmarket surveillance (e.g., surgical drapes, wheelchairs). Class III devices are those that sponsor or sustain life and require premarket approval.
2. In the EU, medical devices are regulated by the EMA under Regulation (EU) 2017/745 and are divided into four categories (I, IIa, IIb, and III) with ascending risk, as per the U.S. system.
3. In Canada, medical devices are regulated by Health Canada under the Medical Devices Regulations (SOR/98-282) as part of the Food and Drugs Act and are divided into four categories (I, II, III and IV) broadly equivalent to the EU categories.

3.2 Cosmetics

Cosmetics are defined by the FDA as products "...intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting the body's structure or functions ...." Many cosmetic products claim to have bioactive ingredients that affect the body's structure and functions (e.g., antiaging and antiwrinkle effects) and the neologism cosmeceuticals has even been proposed for such products, but there is no official recognition by the regulatory authorities. Even so, molecular farming can be used to produce human bioactive proteins such as growth factors and cytokines as cosmetic ingredients, as well as structural skin proteins such as collagen and elastin, which are marketed as cosmetics due to the lower regulatory burden [35].

1. In the USA, cosmetics are regulated by the FDA under the Food, Drug, and Cosmetic Act and the Fair Packaging and Labeling Act. Most cosmetic products and ingredients do not need FDA premarket approval (color additives are an exception) but their safety must be substantiated.
2. In the EU, the manufacture, labeling, and supply of cosmetics and personal care products are regulated by Regulation EC 1223/2009. Manufacturers, retailers and importers are responsible for ensuring all products comply with regulations including the preparation of a Product Information File including the Cosmetic Product Safety Report.

3. In Canada, cosmetics are regulated by Health Canada under the Food and Drugs Act, Cosmetic Regulation and Consumer Packaging and Labelling Act. The Food and Drugs Act sets the definition for a cosmetics product ("... any substance or mixture of substances, manufactured, sold or represented for use in cleansing, improving or altering the complexion, skin, hair or teeth and includes deodorants and perfumes ...") often based upon the manufacturer's claims. The primary requirement is consumer safety, and Health Canada maintains a list of excluded substances that cannot be (deliberately) included in cosmetic products. Cosmetic products placed on the Canadian market, regardless of origin, have to be notified to Health Canada using the Cosmetic Notification Form (CNF) within 10 days of being placed on the market.

3.3 Veterinary Products

Like other product categories, veterinary products are regulated by overlapping legislation governing the production system and the product itself. For products such as veterinary vaccines derived from plant cell suspension cultures (*see* **Note 14**) the rules are identical to those covering other biological products produced by microbes or animal cells. For the products of transgenic plants or transient expression systems in whole plants, the production system plays an increasingly dominant role [36].

1. In the USA, vaccine safety and efficacy are regulated by USDA-APHIS, the same organization that regulates the growth of transgenic plants in containment and in the field, the use of transient expression systems, and the commercial release of transgenic varieties. USDA-APHIS also regulates the use of veterinary products in animal feed, with input on safety from the FDA because the FDA is responsible for regulating the safety of food products from treated animals, such as meat, milk, and eggs. The collection of regulatory responsibility for vaccine production under one organization, and the regulation of safety by the FDA, provides less opportunity for regulatory delays caused by conflicting advice or lack of communication between agencies.
2. In the EU, member state authorities regulate the growth of transgenic plants in containment or in the field. Transient expression systems are not explicitly covered by the regulations but are generally handled as per the rules on GMOs due to the use of recombinant bacteria and/or viruses. If a variety is commercially released, the member state authorities receive an opinion from EFSA on safety but they are not bound by this decision and can unilaterally veto approval. The safety of the product itself is governed by EMA, in consultation with member state authorities. EFSA is responsible for advising on the safety of products added to animal feed, and EMA is

responsible for the safety of products derived from the treated animals. Despite the potential for delays caused by the multiple roles of EMA and EFSA, progress toward approval is facilitated by the key role of the member state competent authorities.

3. In Canada, the regulation of veterinary products is mostly handled by the CFIA although different departments are responsible for different aspects of regulation. The growth and commercial release of transgenic plants is overseen by the CFIA Plant Biosafety Office with input from ECCC if there are specific environmental concerns. Vaccine safety and efficacy are handled by the Canadian Centre for Veterinary Biologics (CCVB), with input from the Animal Feed Division if the product is added to animal feed. The safety of food products from treated animals is regulated by Health Canada.

3.4 Research Reagents

Many molecular farming products are intended as research reagents or diagnostics and these are not regulated at the product level. They may be subject to elective standards introduced by the manufacturer as part of quality control/quality assurance, but statutory regulations only come into play if the reagents are marketed as kits, whereupon they fall under the medical device regulations described above. However, there is a loophole that allows manufacturers to classify diagnostic tests as "laboratory developed tests" provided as a service [37]. New legislation in the EU should close the loophole in 2022 (*see* **Note 15**), whereas similar efforts by the FDA have been only partially successful and Health Canada has thus far taken no action [38]. Other molecular farming products that are not intended for human use, such as industrial enzymes, essentially escape regulation at the product level, but may nevertheless fall within the scope of environmental regulations, as discussed below.

4 Environmental Aspects of Molecular Farming

Disregarding the influence of differing containment practices on product quality and consistency, containment is also important for the regulation of molecular farming in a purely environmental context. In all jurisdictions, molecular farming platforms that are completely contained (plant cell cultures and similar systems in bioreactors) are not subject to any additional regulations than those applying to other cell-based production systems, namely the general practices to maintain safety when handling, using and disposing of GMOs. Similarly, molecular farming in whole plants does not attract any additional regulatory burden if the plants are grown in a greenhouse with adequate systems in place to prevent dissemination of pollen and/or seed into the environment. They are regulated by USDA-APHIS in the USA, individual member

states in the EU, and by ECCC in Canada, which set the minimum specifications for what is considered to be a contained facility. We can therefore focus on the regulations covering the deliberate release of GMOs into the environment, which apply to all transgenic plants. There is a massive gulf in the regulatory frameworks of countries such as the USA and Canada, which regulate biotechnology-derived plants based on the evaluation of risk, and countries/blocs such as the EU, which exercise the precautionary principle and seek evidence for the absence of risk [39, 40].

1. In the USA, molecular farming in whole plants tends to be restricted to greenhouses on an elective basis because it is a more straightforward pathway to commercialization. However, the cultivation of transgenic plants producing pharmaceutical proteins is possible in the open field, and at least one company (Ventria BioScience) has plots of transgenic rice expressing product candidates such as lactoferrin and lysozyme growing in Colorado and on the U.S. Virgin Islands. Both proteins can be used as research reagents, cosmetic ingredients or pharmaceuticals depending on the intended downstream application.
2. USDA-APHIS is responsible for the regulation of field trials and also for the authorization of new transgenic plant varieties for commercial release. Additional regulations imposed by USDA-APHIS to allow open-field cultivation in the USA include precautions to prevent cross-pollination and seed dispersal, which in the Ventria plots is addressed by the choice of crop (rice is a self-fertilizing crop and has no wild relatives within hundreds of miles of the production plots). Recent changes to the SECURE Rule are likely to ease the regulatory burden on outdoor molecular farming crops in the USA even further (*see* **Note 16**).
3. In the EU, molecular farming in whole plants is restricted to greenhouses in a de facto manner even if outdoor cultivation is permitted de jure albeit with heavy regulation. The regulatory burden arises from the strict coexistence legislation, which makes the grower wholly responsible for any pollen or seed making its way to surrounding plots [41]. Molecular farming has not been singled out as a victim of this strict regulation, it applies to all GMOs released into the environment including genetically modified food crops (cultivated widely elsewhere and legally imported into the EU as food and feed products) and even genome edited crops, which in other jurisdictions are classed in the same category as crops developed through conventional breeding if there is no exogenous genetic material. The safety of transgenic plants in the environment is determined by EFSA, but there is no binding mandate on member states to follow EFSA's advice and grant approval for cultivation.

4. In Canada, transgenic plants in the environment fall under the control of the ECCC and the Plant Biodiversity Office of the CFIA, the latter responsible for both field trials and commercial approval. No molecular farming crops had been approved for commercial field production in Canada as of the end of 2020. Field trials of molecular farming crops have several additional conditions imposed over and above those applied to other transgenic crops classed as plants with novel traits (PNTs). These include larger isolation distances, additional toxicity and allergenicity data reviews, and the presence of a CFIA inspector to witness the disposal and destruction of residual plant material. Genetically modified plants are also covered under the New Substances Notification Regulations (Organisms), which require notification of the creation or import of new living organisms (defined as those not included on the Canadian Environmental Protection Act 1999 (CEPA) domestic substances list). ECCC and Health Canada assess the notification to determine whether there is a suspicion of toxicity, which may result in the imposition of additional measures to manage risks to the environment or human health.

5 Conclusions

Molecular farming is the use of plants or other plant-based systems for the production of recombinant protein products that are either purified from plant tissues or used as part of crude preparations or extracts. The regulation of such products depends on their intended applications, which range from research reagents and industrial enzymes (limited regulations) to pharmaceutical products (strict regulations). Production using GMOs is regulated separately depending on the degree of containment, with the strictest regulatory oversight for production systems based on open-field cultivation. This product × process matrix adds a layer of complexity to the regulation of molecular farming products which is not present for traditional manufacturing platforms such as mammalian cells and microbes in bioreactors. Another layer of complexity is added by the disharmony in regulatory frameworks across international boundaries and the various approaches taken in different jurisdictions to account for emergency and compassionate use of unapproved pharmaceuticals, particularly since the advent of COVID-19. In this chapter we have considered the processes required for the approval of molecular farming products representing different product categories and manufacturing processes in three jurisdictions (the USA, the EU, and Canada) in order to provide a broad overview of the regulatory landscape. However, the regulations are constantly shifting and the reader is advised to consult the relevant guidance documents published by the regulatory authorities to provide up to date advice.

6 Notes

1. The definition can also be expanded to include the production of small molecules (usually secondary metabolites with bioactive properties) but in this chapter we restrict the definition to proteins because this is what is covered by current regulations.
2. Regulations have also been developed for nonclonal animal systems, including primary cells, vaccines produced by amplification in chicken eggs, and recombinant proteins produced in the milk of farm animals [42].
3. Living modified organisms (LMOs) are defined in the Cartagena Protocol on Biosafety as living organisms or entities (such as viruses) that contain a novel combination of genetic material generated using modern biotechnology. In general use, LMO is synonymous with genetically modified organism (GMO) as used in the EU, the USA, and Canada, although in the strict sense it must be possible to propagate LMOs, whereas the term GMO may also refer to dead or inactivated biological entities that would be LMOs if alive or active [43].
4. This is generally true, but there are some aspects of a product that would influence the regulatory content. For example, the purification of research reagents from plant tissue is readily achieved using fusion tags such as His_6 or FLAG, but these would be frowned upon in a pharmaceutical context due to potential immunogenicity and unanticipated interactions. More recently, tags have been developed that are compliant with GMP, such as the C-tag [44].
5. Proteins and peptides until recently required an NDA, but the rules were changed following the implementation of the Biologics Price Competition and Innovation (BPCI) Act in 2020. All biological products previously approved with an NDA are deemed to have an applicable BLA under Section 351 of the PHS Act.
6. The definition of what constitutes an orphan disease varies by jurisdiction. The FDA defines it as any disease affecting fewer than 200,000 persons in the USA. EMA defines it as any disease affecting no more than 10,000 people in the EU, although this is not rigorously applied. The two systems are not integrated and diseases may be classed differently in each system. The Canadian system is flexible and is regulated under the Food and Drugs Act and Part C of the Food and Drug Regulations, with approvals granted if the benefits are deemed to outweigh the risks.
7. For transient expression systems, the plants are not transgenic and no specific containment measures are necessary before

infiltration, but it is still advantageous to grow the plants in containment to prevent contamination (e.g., by pests and rodents) and to ensure consistent growth conditions. Importantly, the bacterial suspension used for infiltration must be prepared under GMP conditions including the development of master and working cell banks to ensure consistency between batches.

8. The automation of plant handling can ensure constant and optimized conditions for growth and product accumulation. For transient expression, even the infiltration step can be automated. Automation also eliminates the need for human handling, which reduces the risk of contamination.
9. In the case of plants, general monitoring of the environment can be complemented with image-based monitoring of individual plants—this can be useful to detect infected plants (which can be removed to avoid losing an entire batch) as well as plants suffering poor nutrition.
10. Molecular farming in transgenic plants usually leaves the product trapped, either inside the plant cell or in the apoplast (products secreted from plant cells are often trapped under or within the cell wall). However, it is possible to secrete proteins from the roots of transgenic plants (or hairy root cultures) allowing direct capture from the hydroponic medium [45].
11. Because most molecular farming products accumulate in plant tissues they must be liberated, in many cases by homogenization, which releases a large amount of host cell protein and other impurities. The DSP steps of molecular farming have been adapted to accommodate this, for example by using serial filter trains to remove particulates efficiently [46], the use of flocculants to remove fines by clumping them into larger particles [47], and various strategies to precipitate the bulk of host cell proteins such as heat treatment, pH adjustment, or both [48].
12. Alkaloids such as nicotine should be removed during the processing of tobacco tissue, although low-nicotine varieties may make such steps unnecessary in the future.
13. Plants are often lauded as superior to bacterial production platforms because the former do not produce endotoxins. However, it should be noted that transient expression fills the spaces of the leaves with highly stressed *Agrobacterium tumefaciens*, which respond by producing endotoxins [49].
14. Dow AgroSciences and Fraunhofer IME developed a poultry vaccine against Newcastle disease virus based on stably transformed tobacco BY-2 cells. The vaccine was approved by the USDA Center for Veterinary Biologics but never marketed because it was intended as a means to gauge the reaction of the regulatory system to a new platform technology.

15. Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision. 2017.
16. In May 2020, APHIS revised legislation covering the interstate movement or release of GMOs into the environment (SECURE Rule revision of 7 CFR 340) to make it easier to develop crops with certain types of modifications, including genome edited crops with mutations (as opposed to foreign transgenes) and transgenic crops carrying genes from the same species.

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