CHAPTER

12 RECOMBINANT DNA SAFETY CONSIDERATIONS IN LARGE-SCALE APPLICATIONS AND GOOD MANUFACTURING PRACTICE

Standards should not be forced down from above but rather set by the production workers themselves.

-Taiichi Ohno, Japanese industrial engineer, author of "Toyota Production System: Beyond Large-scale Production"

CHAPTER OUTLINE

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AquAdvantage Salmon.

(Photo courtesy: AquaBounty Technologies)

12.1 INTRODUCTION

Within 3 years of the creation of the first recombinant DNA molecule, the first **biotechnology industry**, Genentech, was founded by Herbert Boyer and Robert Swanson to commercially exploit the technology (see Chapter 4: Recombinant DNA Technology and Genetically Modified Organisms). The initial products of this industry were proteins of pharmaceutical importance (for instance, insulin and human growth factor) obtained by cloning human genes for the proteins in bacteria. With further development of the technology, transgenic plants [genetically modified (GM) crops such as GM soybean, maize, or canola] and transgenic animals (such as the AquAdvantage$^{(R)}$ salmon and RIDL mosquitoes of Oxitec) were developed for commercial cultivation and have been released (or are awaiting regulatory approval for release) into the environment (see Chapter 4: Recombinant DNA Technology and Genetically Modified Organisms). Although risk analysis as part of the regulatory requirement for commercial release of GM organisms (GMOs) ensures that these organisms pose minimal risk to humans, animals, and the environment (see Chapter 10: Risk Analysis), care needs to be exercised while handling these organisms. This chapter discusses Good Industrial Large-Scale Practice (GILSP) and Good Manufacturing Practice (GMP) as applied to GM microbes, plants, animals, and products derived from GMOs.

12.2 SAFETY CONSIDERATIONS FOR INDUSTRIAL APPLICATIONS OF ORGANISMS DERIVED BY RECOMBINANT DNA TECHNIQUES

The first product from a GMO was marketed in 1982 (Humulin, human insulin expressed in bacteria, developed by Genentech and licensed to Eli Lilly and Co.) and was soon followed by a number

of other pharmaceutically important proteins (see Chapter 4: Recombinant DNA Technology and Genetically Modified Organisms). Production volumes of over thousands of liters of bacterial culture could be achieved through **extensive experience of scale-up from laboratory level, to pilot scale, and finally, manufacturing levels of foods by fermentation processes using bacteria, yeasts, and fungi**. Traditional fermentation processes in industries (such as the beer, wine, cheese, and other fermented food industries) use microorganisms that are well characterized and considered to be of low-risk. Therefore, such industries require only minimal controls and containment procedures. When rDNA was first introduced, the major concern was regarding potential hazards, such as allergenicity, toxicity, or other effects, on humans and animals. In order to avoid persistence of escapees in the environment, **initial research and production systems using rDNA technology were confined to a strain of gut bacteria, *Escherichia coli* K12**. This strain had been cultured in laboratories for several decades and had lost several genes present in the wild-type strains of *E. coli* necessary for colonizing the human gut. These included the cell surface K antigen, part of the lipopolysaccharide side chain, resistance to lysis by complement in human serum and to phagocytosis by white blood cells, and an adherence factor that enabled the bacteria to stick to epithelial cells of the gut. The *E. coli* K12 bacteria were also incapable of synthesizing certain ingredients necessary for growth (had to be supplied in the culture medium) or repairing DNA by recombination (hence, readily killed by exposure to ultra violet rays present in sunlight). In short, **the *E. coli* K12 strain was incapable of causing allergies, disease, or survival outside the laboratory**.

Pioneering efforts in understanding safety issues in the nascent biotech industry and in implementing processes that would ensure safety in the application of rDNA technology were made by the Ad hoc Group of government experts created by the Committee for Scientific and Technological Policy of the **Organization for Economic Cooperation and Development** (OECD; http://www.oecd.org/). In 1986, OECD Council decided to make public a report prepared by the Ad hoc Group and to adopt recommendations made in the report to ensure safety in applications of rDNA organisms in industry, agriculture, and the environment (OECD, 1986). The report established a concept of **GILSP** applicable to **low-risk organisms used in industrial production**. Key concepts to GILSP are as follows:

1. Risk assessment of the recombinant organism to ensure that it is as safe as the low-risk host organism
2. Identification and adoption of safe practices.

12.3 GOOD INDUSTRIAL LARGE-SCALE PRACTICE

Potential risks to the environment of the applications of rDNA organisms are minimized due to a "**step-by-step assessment during the research and development process,**" that is from laboratory scale, to pilot scale level, to finally, industrial level. For rDNA microorganisms and cell cultures, the **criteria for GILSP** suggested by the OECD include the following:

- **Host**:
  - ***Nonpathogenic***: Hosts containing the recombinant nucleic acid should be identified and established to be nonpathogenic. They should also not produce any toxins or allergens.
  - ***No adventitious agents***: The hosts should not harbor any viruses or mycoplasma.

  - ***Extended history of safe use***: Sufficient documented experience of safe use of the host organism should be available. Safe use could also be established by laboratory/pilot-scale fermentations under conditions of minimal containment.
  - ***Built-in environmental limitations***: Should permit optimal growth in industry but limited survival in the environment, such as strains sensitive to ultraviolet light or requiring supplements in growth media of substances not found in nature. Any surviving microbes should have minimal adverse environmental consequences.
- **Vector/Insert**:
  - ***Well characterized and free from known harmful sequences***:
    - ***Vector***: Knowledge of the derivation and construction of the vector and subsequent experimental confirmation of the construct is necessary to ensure that the vector is free from sequences that result in phenotypes harmful to humans or the environment such as production of toxins or factors that promote pathogenicity.
    - ***Insert***: Source and function of the DNA being inserted and the point of insertion should be known.
  - ***Limited in size***: The vector/insert should be as limited in size as possible in order to decrease the probability of carrying unwanted genes and other sequences.
  - ***Should be poorly mobilizable***: The rate at which it may be transferred from the original recipient to other organisms should be low, for example, by eliminating transfer functions of plasmids, or by integration into host chromosome.
  - ***Should not transfer any resistance markers to microorganisms not known to acquire them naturally***: Genetic markers conferring resistance to substances, such as antibiotics, herbicides, or heavy metals, are often used in rDNA technology to select the transformed organisms from untransformed hosts. Use of these markers should take into consideration the possibility of spread and the impact of the marker on the environment.
- **rDNA organism**:
  - ***Nonpathogenic***: The rDNA organism is expected to be nonpathogenic as the gene product has no known role in pathogenicity and the host is nonpathogenic.
  - ***As safe in an industrial setting as host organism***: The rDNA organism should have limited survival or have no adverse consequence to humans and the environment.

The report recognized that there may be some circumstances under which physical containment may be warranted, as when pathogenic organisms are used, or genes coding for harmful products are inserted. Under such circumstances, industry safety programs rely on the two approaches of:

- **biological containment**—takes advantage of natural barriers that limit the survival and multiplication of the organism in the environment, and/or transmission of the genetic information to other organisms;
- **physical containment**—which uses (1) equipment, (2) operating practices, and (3) design of facility to protect personnel handling the organisms and the environment outside the facility.

Physical containment for large-scale uses of organisms containing recombinant or synthetic nucleic acids have been addressed in Appendix K of the guidelines proposed by the National Institutes of Health (see Box 12.1).

BOX 12.1 NIH GUIDELINES: PHYSICAL CONTAINMENT FOR LARGE-SCALE APPLICATIONS OF ORGANISMS CONTAINING RECOMBINANT OR SYNTHETIC NUCLEIC ACIDS

Appendix K of the NIH guidelines (NIH Guidelines, 2016) specifies physical containment guidelines for addressing the biological hazard associated with research or production involving greater than 10 L of culture of viable organisms containing recombinant or synthetic nucleic acid molecules. Appendix K supersedes Appendix G, *Physical Containment*, in cases when culture volumes are in excess of 10 L. The guideline establishes four levels of physical containment commensurate with the assessed degree of hazard to health or to the environment posed by the organism based on experience with similar organisms that have not been modified, and on GILSP. The four levels of containment are referred to as Good Large-Scale Practice, BL1-Large Scale, BL2-Large Scale, and BL3-Large Scale.

Good Large-Scale Practice: This level is recommended for large-scale research or production involving organisms that are generally regarded as safe, nonpathogenic, nontoxigenic, and derived from host organisms that have an extended history of safe use. In order to ensure safety, measures taken include the following:

- Institutional codes of practice shall be formulated and implemented
- Personnel are trained to handle modified organisms
- Basic hygiene and safety measures such as hand washing, prohibition of eating, drinking, and smoking in work area
- Discharges containing viable organisms are treated as per environmental regulations
- An emergency response plan shall include provisions for handling spills.

Biosafety Level 1 (BL1)-Large Scale: This level is recommended for organisms that qualify for BL1 containment at the laboratory scale and do not qualify for Good Large-Scale Practice.

- Spills and accidents that result in exposure to the modified organisms are immediately reported to the Laboratory Director. Medical evaluation, surveillance, and treatment are provided as appropriate and documented.
- Cultures of viable modified organisms shall be handled in a closed system (e.g., closed culture vessels)
- Culture fluids shall not be removed from the closed vessel unless viable organisms have been inactivated
- Exhaust gases shall be treated by filters
- Emergency plans including handling large losses of culture on an emergency basis required by the Institutional Biosafety Committee and Biological Safety Officer.

Biosafety Level 2 (BL2)-Large Scale: This level is recommended for organisms that qualify for BL2 containment at the laboratory scale.

- Spills and accidents that result in exposure to the modified organisms are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH Office of Science Policy, and other appropriate authorities. Medical evaluation, surveillance, and treatment are provided as appropriate and documented.
- As in BL1-Large Scale, closed systems shall be used for the propagation and growth of viable modified organisms, which shall not be opened unless sterilized by a validated procedure.
- A sign with the universal biosafety symbol shall be posted on each closed system and primary containment equipment.
- Emergency plans including handling large losses of culture on an emergency basis required by the Institutional Biosafety Committee and Biological Safety Officer.

Biosafety Level 3 (BL3)-Large Scale: This level is recommended for organisms that qualify for BL3 containment at the laboratory scale.

- As in BL1-Large Scale and
- The controlled area shall have a separate entry, double door with airlocks, or change room separating the controlled area from the rest of the facility. A shower facility shall be provided. Entry to the controlled area shall be restricted to authorized personnel only and will be only through the double doors.

(*Continued*)

BOX 12.1 (CONTINUED)

- An effective insect and rodent program shall be maintained; the controlled area shall be decontaminated in accordance with standard procedures in the event of a spill or accident.

Currently, organisms that require BL4 containment in the laboratory scale are not permitted for large-scale applications.

Reference

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), 2016, Retrieved from http://osp.od.nih.gov/sites/default/files/NIH_Guidelines_0.pdf.

An internal survey carried out in the OECD countries in 1988 revealed that the underlying principles of the GILSP concept had been adopted in national guidelines in several countries and was being considered for implementation by others. In 1992, the OECD brought out an updated follow-up to the 1986 publication (OECD, 1992). This document introduced the concept of "**Good Developmental Principles**" (GDP) for the design of small-scale field research with plants and microorganisms with newly introduced traits.

KEY TAKEAWAYS

Criteria suggested by OECD for rDNA GILSP microorganisms and cell cultures are as follows:

- **Host organism—**should be nonpathogenic, should not harbor adventitious agents, should have extended history of safe use or built-in environmental limitations
- **Vector/Insert—**should be well characterized, free from harmful sequence, as limited in size as possible, poorly mobilizable, should not transfer resistance markers to microorganism not known to acquire them naturally
- **rDNA organism—**should be nonpathogenic, safe in industrial settings, limited survival with minimal adverse effects in environment.

12.4 GOOD DEVELOPMENTAL PRINCIPLES

The development of a GMO for use in the environment generally goes through **three stages**. The **first stage consists of experiments done in the laboratory and the glasshouse/greenhouse**. Both nationally and internationally, codes have been developed for ensuring safety under laboratory conditions. But the OECD's Group of National Experts on Safety in Biotechnology felt that it was necessary to "*develop general principles that would identify a generic approach to the safety assessment of low—or negligible risk* ***small-scale field research***" (OECD, 1992) **which represents the second stage of development**. **The third stage of development is the release of the variety/production**. The GDP were developed to address this need.

KEY TAKEAWAYS

Three stages of product development are as follows:

- **Stage 1**—Laboratory/greenhouse
- **Stage 2**—Basic field research and small-scale field research (***principles of GDP applied here***)
- **Stage 3**—Applied large-scale field trials and production/release.

Key safety factors identified for ensuring safety in experiments include:

1. ***The characteristics of the organism***: That is, the introduced gene/genetic material. In many cases, the organism may be safe under a wide range of environmental conditions, but it could be possible to grow organisms known to cause adverse effects under confinement or by exercising mitigation methods.
2. ***The characteristics of the research site***: Research sites selected for field trials should meet the objectives of the experiment and should take into account important ecological and/or environmental considerations related to safety; the climatic conditions; appropriateness in terms of geographical location and proximity to specific biota that may be affected; and the size of the site.
3. ***The experimental conditions***: In order to obtain scientifically acceptable and environmentally sound data, the experiment should be designed carefully. This necessitates attention to the formulation of a hypothesis and statement of objectives, precise experimental protocols including planting density and treatment patterns, collection, and analysis of experimental data to draw conclusions based on statistical significance.

The principles of GDP facilitate the design and conduct of field experiments so that

- the experimental GM plants remain reproductively isolated from unmodified plants grown outside the experimental area
- GMOs or their genes will not be released into the environment beyond the experimental site, or
- even without reproductive isolation, the plants will not cause unintended, uncontrolled adverse effects.

12.5 SAFETY CONSIDERATIONS FOR FIELD/MARKET RELEASE OF GMOs AND/OR THEIR PRODUCTS

Recombinant DNA technology has been used to produce food, industrial chemicals, and medicinal products from GM microorganisms, cell lines, plants, and animals. Safety considerations for each application are distinct and dependent on the host, the gene transferred, and the application itself. Discussed in the following sections are the issues associated with each category of applications:

12.5.1 SAFETY CONSIDERATIONS FOR FIELD RELEASE OF GENETICALLY MODIFIED (GM) CROPS

Commercial cultivation of GM crops began in 1986 with regulatory approval being given for the cultivation of herbicide resistant tobacco. Since then, GM crops have been grown in 28 countries in

around 180 million hectares. GM varieties exist for major crops such as soybean, maize, canola, cotton and are being developed for rice and several others (see Chapter 4: Recombinant DNA Technology and Genetically Modified Organisms). Risk analysis of the GM crop and of foods derived from such crops conducted as part of the regulatory process ensures that commercially cultivated GM crops pose no safety issues to humans, animals, or to the environment (see Chapter 10: Risk Analysis). However, one category of GM plants that **warrant special consideration regarding biosafety are plants modified for the purpose of producing recombinant proteins for pharmaceutical or industrial use (plant molecular farming (PMF)**, also known as pharming, see Chapter 4: Recombinant DNA Technology and Genetically Modified Organisms). This type of application raises concerns regarding aspects of **transgene spread in the environment**, or **accidental contamination of the food/feed chains as these plants are not meant for food/feed use**. Host systems used in PMF include **food plants** (such as maize, soybean, potato, oilseed rape, tomato, banana, and rice), **nonfood plants** (such as tobacco), **noncultivated plants** (such as duckweed, *Arabidopsis*), and **cultured plant cells** (such as carrot, tobacco, and tomato). **The choice of production platform has a significant impact on biosafety in PMF**.

The use of food crops as production systems has been particularly controversial due to the **risk of GM crops inadvertently entering the food chain**. An instance illustrative of the problem of accidental contamination occurred in 2002 when farmers in Nebraska planted conventional soybeans for human consumption in a field previously used to test GM maize producing a pig vaccine by a biotech company, ProdiGene. US Department of Agriculture (USDA) inspectors found 500,000 bushels of soybean contaminated with GM maize stalks and leaves as the firm had neglected to remove volunteer corn plants that sprouted alongside the soybean. ProdiGene was ordered to pay a US$ 250,000 fine, and buy and destroy all the contaminated soybean. Earlier that year, the company had been asked to destroy 155 acres of corn in Iowa contaminated with GM corn producing Trypsin (pancreatic serine protease), not approved for consumption by humans or animals (Fox, 2003). **The USDA has since enforced a zero-tolerance standard, whereby plants grown for pharmaceutical or industrial products (and not approved for food/feed) have to remain distinct from the food system** (USDA, 2006). Many countries recommend the use of nonfood plants or cell cultures for pharming.

Physical and biological containment may be considered on a case-by-case basis as a viable option to limit adverse impacts on the environment or contamination of food/feed systems (Breyer et al., 2009) as discussed below:

Physical containment:

Several plants, such as tobacco, potato, and tomatoes, can be grown in glasshouses, greenhouses, plastic tunnels, and other forms of physical containment. Although this option is effective in preventing contamination of food systems, practical difficulties include **issues of scale-up** and **additional financial resources**.

Spatial containment:

This option aims to minimize pollen transmission of traits from the GM to non-GM crops while being more flexible in terms of scale-up. Strategies used include the following:

- *Dedicated land*: Pharming is conducted in regions where similar crops are not grown or locations considerably distant from nonmodified crops so as to eliminate the risk of gene flow. This option is not always feasible due to unfavorable agroclimatic conditions.

- *Restricted use*: This option is to restrict pharming to a designated area for a specified number of growing seasons, during which nonmodified crops for food/feed would not be grown.
- *Buffer and border zones*: Pharming could also employ strategies used to grow other GM crops. A minimum isolation distance (buffer zone) which depends on the biology of the crop plant (self-pollinated/wind/insect pollinated) could be set up around the GM crop. Alternatively, borders of non-GM plants could be planted around the GM crop stand to "trap" pollen from the GM plants. These strategies may not, however, ensure zero contamination.

Biological containment:
Several strategies based on many different biological principles have been suggested:

- *Plastid transformation*: Here, the transgene is inserted in the chloroplast genome rather than the nuclear genome. Several advantages of this technique include the ability to control gene insertion more precisely, higher rates of transgene expression and protein accumulation, but most significantly, in higher plants, it prevents pollen transmission of the transgene as pollen grains lack chloroplasts.
- *Male sterility*: Natural and induced male sterility has been used by plant breeders to control crossing and may be used to prevent pollen transmission of modified genes.
- *Genetic Use Restriction Technologies (GURT)*: Although this genetic system has been much criticized by the social media as being a technique developed by multinational seed companies to control the seed market and to enforce intellectual property rights, GURT could be used to ensure that the trait is not carried forward to the next generation either because the seeds are sterile (as in V-GURT or "terminator technologies") or the trait is not expressed in the progeny (as in T-GURT or "traitor technologies") unless treated with an inducer (see Chapter 5: Relevance of Ethics in Biotechnology).

Other Biological Containment Strategies:
Several other containment mechanisms may be developed in future which exploit natural mechanisms, such as apomixes (production of seed without pollination); cleistogamy (self-pollination and fertilization within flowers that are closed); or targeted/spatial and temporal gene expression so the expressed products are present only in specific organs such as roots, seeds, or edible plant parts.

Temporal confinement:
Temporal confinement can be achieved by either physical or biological methods, such as timing the crop for PMF at different times to prevent overlap with the food/feed crop; or to have only transient expression of the introduced genes (as the gene is not stably integrated in the host genome, it will not be heritable).

Additional considerations:
In order to avoid issues of contamination of nonmodified plant products with products from pharming, care is to be exercised in the handling and transport of products, the cleaning of equipment (preferably, dedicated equipment to be used), and the personnel employed. Also warranting attention is waste management: residual material left on the field/storage areas and the by-products of the processing. Adequate postmarket management measures such as inspection of the cultivation site and monitoring of the product is necessary to ensure that no adverse effects occur.

KEY TAKEAWAYS

Safety considerations for field release of GM crops for nonfood products to prevent contamination of food/feed include the following:

- ***Physical containment***: growing plants in glasshouse, greenhouses, plastic tunnels
- ***Spatial containment***: minimizing pollen transmission of traits from modified to non-GM crops
  - ***Dedicated land***
  - ***Restricted use***
  - ***Buffer/border zones***
- ***Biological containment***:
  - ***Plastid transformation***: prevents pollen transmission of traits
  - ***Male sterility***
  - *GURT*
  - ***Other Biological techniques***: apomixes, cleistogamy, targeted spatial gene expression
- ***Temporal confinement***: timing the modified crop to prevent overlap with the food/feed crop, transient gene expression.

12.5.2 SAFETY CONSIDERATIONS FOR FIELD RELEASE OF GENETICALLY MODIFIED ANIMALS

Genetically engineered animals have biomedical applications, such as production of human proteins, drugs, vaccines, and replacement tissues, as well as applications in agriculture, such as production of food and animal welfare, in addition to reducing the impact on the environment due to better utilization of resources (Gottlieb & Wheeler, 2011). Management practices employed to mitigate assessed environmental risks of transgenic animals include maintaining the animals in specialized facilities that minimize contact with people, other animals, insects, and infectious agents. There have been till date, **no GE animals placed in the market in the European Union**, but in the United States, the **Food and Drug Administration (FDA)** has approved several **transgenic animals for the commercial production of pharmaceutical compounds and for food uses**. These include:

- goats that produce an anticoagulant, ATryn (antithrombin), in milk (2009)
- rabbits that produce a drug for treating angioedema (2014)
- AquAdvantage salmon for food (2015)
- Chicken that produce a drug kanuma (sebelipase alfa) in eggs (used to treat people with a rare genetic condition that prevents the body from breaking down fatty molecules in cells) (2015)

In the pipeline are GM mosquitos, *Aedes aegypti* (OX513A), produced by Oxitec for vector control strategy for preventing mosquito-borne viral diseases including Zika, dengue, chikungunya, and yellow fever. Although the FDA released the final Environment Assessment submitted by Oxitec based on a field trial conducted in Florida Keys and the final Finding of No Significant Impact on August 5, 2016, the GE mosquitos are yet to be approved for commercial use (FDA, 2016).

The FDA places the onus of ensuring safety in commercial use of transgenic on the sponsor. In the case of the GE mosquitoes, the company Oxitec is responsible for ensuring all local, state, and federal requirements met before conducting field trials. The company AquaBounty Technologies which produces AquAdvantage salmon has put in place physical and biological containment strategies to ensure that the modified salmon does not impact natural salmon population (see Box 4.1).

Containment strategies for GM animals would be concomitant with the phenotype of the animal, the nature of the activity, and the assessed risk due to the modification. Physical containment should prevent animals from escaping into the wider environment and will typically consist of pens, cages, and other enclosures. Double fencing may sometimes be appropriate given the level of risk. Aquatic animals should be kept in tanks fitted with filters sufficient to prevent escape of eggs or the smallest fingerlings. Access to the containment facility should be restricted and monitored. Disposal of waste and carcasses from the facility should be handled with care. Additional biological containment could be effected by reproductive sterility (e.g., by polyploidy as in the case of AquAdvantage salmon or genetic sterility as in the case of the RIDL mosquitos).

KEY TAKEAWAYS

Safety considerations for field release of transgenic animals:

- ***Physical containment***: pens, cages, water tanks with water filters; double fencing; waste disposal done with care
- ***Biological containment***: sterility due to polyploidy or genetic sterility

12.5.3 SAFETY CONSIDERATIONS FOR MARKETING OF FOODS FROM GENETICALLY MODIFIED ORGANISMS

Highly polarized views exist with regard to foods produced from GM plants and animals, with opponents referring to them as "Frankenfoods" and proponents insisting that they are **substantially equivalent to** and inherently **do not pose more risk than foods from unmodified organisms**. Many scientific organizations believe that genetic engineering is merely an extension of breeding techniques and is **no more unsafe as the genetic manipulations of conventional breeding methods.** For instance, the American Association for the Advancement of Science in a statement issued in 2012 pointed out that the EU had invested more than €300 million in research on the biosafety of GMOs and concluded in its report based on more than 130 research projects over 25 years involving more than 500 independent research groups, that biotechnology, in particular **GMOs, are not per se more risky than conventional breeding technologies** (AAAS, 2012). Subsequently, the US FDA issued guidance on voluntary labeling of foods from genetically engineered sources stating that under the federal FD&C Act, "*the FDA can only require additional labeling of foods derived from GE sources if there is a material difference—such as different nutritional profile—between the GE product and its non-GE counterpart*" (FDA, 2015). US polls on GE food labeling show that the majority (89%) favor mandatory labeling with only 6% opposed to it. These views were widespread across demographic lines: Democrats (92% favor, 2% oppose), Independents

(89% favor, 7% oppose), and Republicans (84% favor, 7% oppose) (The Mellman Group, Inc., 2015). On July 29, **2016, President Obama signed into law a bill that will require labeling of GM ingredients being marketed in the United States**—food packages would need to carry a text label, a symbol or an electronic code readable by a smartphone whether the food contains GMOs (Jalonick, 2016). Earlier Vermont state laws had made it mandatory for GMO foods to be labeled as "produced with genetic engineering."

The European Union has enforced consumer "right to know" laws for GM foods through **Regulation (EC) No. 1830/2003** that mandates the **traceability and labeling of GMOs** and of **food and feed products produced from GMOs** (European Union, 2003). Under this regulation, products such as flour, oils, and syrups have to be labeled as GM if derived from GM crops. However, products produced with GM technology (for instance, cheese produced using GM enzymes, as well as meat, milk, or eggs from animals fed on GM feed) do not have to be labeled. Traceability requirements enable tracking GMOs and GM food/feed products at all stages of the supply chain. This means that all operators involved (such as the farmers/producers of food or feed) must provide customers with information regarding the product, or ingredients in the product, having been derived from GMOs. Also, a record of transactions within the supply chain is to be maintained by all operators and made available for a period of 5 years.

Several nations have also introduced "**GM-free**" labels that indicate that specific measures have been taken to strictly exclude the presence or use of GMOs in the food/feed products.

KEY TAKEAWAYS

Safety considerations for GM foods:

- The FDA considers food from GM animals to be not per se more risky than that from conventionally bred animals and only requires voluntary labeling of foods derived from GE sources
- The EU mandates traceability and labeling of GMOs and food/feed products produced from GMOs
- Several nations have introduced "GM-free" labels to indicate that the product contains no GM ingredients.

12.5.4 SAFETY CONSIDERATIONS FOR MARKET APPROVAL OF BIOPHARMACEUTICALS

Biopharmaceuticals [also known as "biologics" or "biologic(al) medicinal products"] are medicinal products manufactured by biotechnology methods from living organisms or their products and include all recombinant proteins, monoclonal antibodies, vaccines, blood/plasma-derived products, nonrecombinant culture-derived proteins, and cultured cells and tissues. Although technical differences exist in the manner in which biologics are regulated in different regions such as the United States and Europe, efforts have been made to harmonize requirements for market approval of this class of medicines to ensure patient safety (Kingham, Klasa & Carver, 2014). This is because

regulatory authorities world over recognize that biologics (unlike chemical drugs) are largely complex in structure and susceptible to variation during manufacture.

In the United States, for market approval, the sponsor of a chemical (nonbiologic) drug must submit a New Drug Application (NDA) that shows that the drug is safe and effective. But in the case of a biological product, the **Biologics License Application (BLA)** must prove that the product is "***safe, pure, and potent.***" This means that for approval biologics must undergo **laboratory and animal testing to define their pharmacologic and toxicologic effects and prove clinical benefit in human clinical trials**. For **nonclinical studies for biologics**, the FDA has adopted the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use **S6 guidelines** (ICH, 2011). In order to conduct clinical tests, the sponsor must first have an **Investigational New Drug (IND) Application** in effect (an IND generally goes into effect 30 days after the application, unless on review the FDA places the trial on hold, for instance because it deems it to place trial patients in unreasonable risk). **The IND should therefore provide sufficient proof of safety in preclinical trials for the conduct of a clinical trial**. The FDA has adopted the guideline **for Good Clinical Practice (E6 guideline)** developed by the ICH (1996) for the conduct of clinical trials in order to protect clinical trial subjects and to ensure the integrity of data collected during the trial (see Section 5.6 in Chapter 5: Relevance of Ethics in Biotechnology). **In addition to the nonclinical and clinical data, the BLA should also contain a full description of manufacturing methods for the product; stability data substantiating the expiration date; product samples along with summary of test results for the batch from which derived; as well as details of address of manufacturing unit, labeling, packaging, and enclosures**.

In Europe, the regulatory authority Committee for Medicinal Products for Human use (CHMP) of the European Medicines Agency (EMA) defines biologics "largely by the method of manufacture." The CHMP has also adopted the **ICH S6 as guideline for preclinical testing** of biologics. Subsequent to the preclinical trials, clinical trials have to be conducted before a **Market Authorization Application (MAA)** can be made. **Clinical trials of biologics must comply with Directive 2005/28/EC on Good Clinical Practice and the ICH E6 guideline** adopted by CHMP. The principles for clinical trials detailed in the directive and guideline ensure that the rights, safety, and well-being of trial subjects take precedence over the interests of science and society and the ethical principles of the World Medical Association's Declaration of Helsinki (see Box 5.1 in Chapter 5: Relevance of Ethics in Biotechnology) are upheld. Under the **Clinical Trials Directive**, information regarding trials must be recorded in the European database of clinical trials accessible only to other competent authorities, the EMA and the European Commission. The MAAs for biologics in addition to providing standard information described in the Medicines Directive, also has **special information requirements**, such as: details of manufacturing process; origin and history of starting materials; should demonstrate that the active substance complies with special measures for preventing transmission of animal spongiform encephalopathies; should demonstrate that cell banks if used are stable; provide information on adventitious agents that may be present; describe origin, criteria, procedures for collection, transportation, and storage of starting material if medicines derived from blood or plasma; base vaccine production on a seed lot system and established cell banks if possible; and describe the manufacturing facilities and equipment. The manufacture of biologics is expected to comply with **GMPs** during all clinical trial phases and after market approval.

KEY TAKEAWAYS

United States:

- BLA must prove product is safe, pure, and potent
- Nonclinical trials to comply with ICH S6 Guideline
- Sponsor should have an Investigational NDA in effect for conduct of clinical trials
- Clinical trials should comply with Good Clinical practice, the ICH E6 Guideline

Europe:

- The CHMP of EMA also adopts ICH S6 for preclinical testing
- Clinical trials must comply with Directive 2005/28/EC and ICH E6 Guideline
- MAA should also have details of manufacturing process for the biologic

12.5.5 SAFETY CONSIDERATIONS FOR MARKET APPROVAL OF BIOSIMILARS

Biosimilars (also known as "follow on biologics" or "subsequent entry biologics") are medicinal products similar to an original "innovator" biologic that can be manufactured when the original product's patent expires. Biosimilars can therefore be produced by different companies and very often use different starting materials as these companies may not have access to the original cell line, or the exact fermentation or purification method used by the originator. In order to be approved for commercial production, companies have to demonstrate to the regulatory authorities that the product is **"similar" in terms of safety and efficacy to the reference product** (hence, the term "biosimilar"). The approval process for biosimilars is not the same as for generic versions of small molecule drugs that are products of easily defined synthetic or semisynthetic processes. As biologics are complex and as mentioned earlier, prone to variations, product quality, and integrity will differ for each manufacturer. Regulatory authorities such as the EMA, the FDA, and Health Canada each have issued specific guidance on the requirements for the approval of biosimilars (Blank et al., 2013). **The approval procedure is based on the demonstration of "comparability" of the structure and function(s), pharmacokinetic profiles and pharmacodynamics effects/efficacy of the biosimilar, to the approved biologic**. Biosimilars, as do biologics, also present some risk of adverse reactions or unwanted immune reactions to the medicine. In order to ensure patient safety, the introduction of biosimilars requires a specifically designed **pharmacovigilance plan**. The EMA needs a risk management plan to be submitted along with the market approval application and requires that the company provides regular safety update reports after the product is in the market. In the United States, a drug approved for marketing has to be revaluated for its safety and efficacy once every 6 months for the first 2 years, and subsequently every year, reports of which are to be filed with the FDA.

12.6 GOOD MANUFACTURING PRACTICES

GMPs are systems that provide proper design, monitoring and control of manufacturing processes and facilities, and thereby assure the identity, strength, quality, and purity of drug products.

They are often referred to as Current GMPs, indicating that manufacturers are to use state-of-the-art, or the most modern technologies and systems. The primary aim of GMP is to diminish risks inherent in production of pharmaceuticals, such as cross-contamination and false labeling.

The first draft text of GMP was prepared in 1967 by the World Health Organization (WHO) and published as an Annex to its 22nd report in 1968. Subsequent developments have resulted in revisions and incorporation of the concept of validation (available online at http://www.who.int/medicines/areas/quality_safety/quality_assurance/production/en/). The main principles of GMP for pharmaceutical products were updated and published in 2014 as **Annex 2** of the **WHO Technical Report 986** (WHO, 2014). Biologics warrant special considerations and **GMP for biologics was first published in 1992**. The current updated version has been published as **Annex 3** of the **WHO Technical Report 996** in 2016 (WHO, 2016). These documents serve as guidance for national regulatory authorities and for manufacturers of pharmaceutical products and could be incorporated into national legal requirements. The GMP for biologics address manufacturing procedures that involve growth of microorganisms and eukaryotic cells; extraction of substances from biological tissues; recombinant DNA and hybridoma techniques; and propagation of microorganisms in embryos or animals. For more details, see Box 12.2.

BOX 12.2 WHO GOOD MANUFACTURING PRACTICES FOR BIOLOGICAL PRODUCTS

The WHO GMP for biological products applies to the manufacture of medicinal products including "*allergens, antigens, vaccines, certain hormones, cytokines, monoclonal antibodies (mAbs), enzymes, animal immune sera, products of fermentation (including products derived from rDNA), biological diagnostic reagents for in vivo use and advanced therapy medicinal products (ATMPs) used for example in gene therapy and cell therapy*" (WHO, 2016). Special considerations and precautions are warranted in the manufacture of these products because unlike other medicinal products manufactured by defined and mostly consistent chemical or physical methods, biologics are derivatives of biological processes which may be inherently variable. Quality Risk Management (QRM) principles are, therefore, especially important to this class of medicines and extend across all stages of the manufacturing process including: material sourcing and storage; manufacture and packaging; quality control; quality assurance, storage, and distribution activities.

Personnel: Only trained personnel with adequate scientific experience should handle the different steps in the manufacture of biologics.

Starting materials: The source, origin, and suitability of active substances starting materials, buffers and media, and other components should be documented and the information retained for at least 1 year after the expiry date of the finished product as it may be useful in investigating adverse events if it occurs. All suppliers should be initially qualified and identity tests performed on each batch of supplies without adversely affecting the quality of the product in order to prevent contamination or cross-contamination. Sterilization of starting material if required should be done with heat whenever possible. Risk of contamination of the starting material during passage through the supply chain should be assessed.

Seed lots and cell banks: Appropriate controls over sourcing, testing, transport, and storage should be exercised when human or animal cells are used as feeder cells in the manufacture process. In order to prevent genetic drift due to passaging, a system of master seed lots or cell banks (MCB) and working cell banks (WCB) should be set up. The number of passages between the seed lot or cell bank and the finished product should be consistent with the marketing authorization application. Establishment and handling of the MCB and WCB should be performed under appropriate conditions, and during establishment, no other infectious agent should be simultaneously handled in the same area or by the same personnel. Appropriate quarantine and release procedures should be followed for the MCB and WCB.

(*Continued*)

BOX 12.2 (CONTINUED)

Also, the seed banks should be handled in a manner as to minimize risk of contamination, alteration, or cross-contamination. Storage and handling conditions should be defined, access to the material restricted to authorized personnel, and records maintained as to location and identity.

Premises and equipment: Quality risk management (QRM) principles should be adhered to in the handling of preparations containing live microorganisms or viruses, which includes avoiding the handling of organisms in areas used for processing of other pharmaceutical products. The use of closed systems to improve sepsis, and containment, should be considered wherever possible. Adequate attention is to be given to cleaning and sanitation measures.

Containment: Airborne dissemination of live microorganisms and viruses including those from personnel is to be avoided. Drainage systems should be designed to allow decontamination or effective neutralization of effluents and minimize risk of cross-contamination. For handling of pathogenic organisms of Biosafety risk group 3 or 4, and/or spore forming organisms, dedicated production areas should be used. Air-handling systems should be designed, constructed, and maintained to prevent crosscontamination between different manufacturing areas. Areas where Biosafety risk group 3 or 4 organisms are handled should always be under negative air pressure.

Clean rooms: The WHO GMP for sterile pharmaceutical products defines and establishes requirements for clean areas for the manufacture and aseptic fill of sterile products. Specific guideline is also available for the production of vaccines. The degree of environmental control of particulate and microbial contamination of the production area would depend on the potential level of contamination in the starting material and risks to the finished product.

Production: Typically biologics would require conditions, media, and reagents that promote growth of cells or microbes in axenic conditions; hence, effective technical and organizational measures are to be taken to prevent contamination and cross-contamination. This includes the design of the facility as well as the processes involved that need to be in keeping with QRM principles.

Labeling: Information to be provided on the container (inner) label as well as the packaging (outer label) should be readable and legible and the content approved by the national regulatory authority. Care is to be taken for the label to remain attached under different storage conditions, including ultralow temperatures of the product.

Validation: The handling of live material, and cleaning, is the major aspects of biological product manufacturing that require validation. It plays an important part in production consistency, control of critical process parameters, and product attributes. A QRM approach is to be adopted to determine the scope and extent of validation.

Quality control: Special consideration is to be given to the nature of the materials being sampled for quality control and testing. Samples for postrelease use belong to two categories: the reference samples and the retention samples, which for finished products may be presented as fully packaged units. Reference samples of biological starting materials should be retained under recommended storage conditions for at least a year. Retention samples of finished product should be stored in their final packaging for at least a year after the expiry date under the recommended storage conditions. The traceability, proper use, and storage of reference standards should be ensured, defined, and recorded. All analytical methods used in quality control of biological products should be well characterized, validated, and documented; the fundamental parameters of validation include linearity, accuracy, precision, selectivity/specificity, sensitivity, and reproducibility.

Documentation (batch-processing records): Processing records of regular production batches should provide a complete account of the manufacturing activities. Manufacturing batch records are to be retained for at least a year after the expiry date of the batch of the biological product.

Use of animals: Animals may be used for the manufacture or quality control of biological products. Live animals are to be avoided in the production area unless otherwise justified. Embryonated eggs if applicable are allowed in the production area. If extraction of tissues or organs is required, special care is to be taken to prevent contamination of the production area. Areas used for performing tests should be well separated from areas used for manufacturing and should have a separate ventilation system. The animals are to be properly housed and care to be taken to prevent and monitor infections.

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12.7 SUMMARY

One of the most significant aspects of recombinant DNA technology and one that has spurred innovation in the field is the possibility to commercially exploit the technology. Safety considerations that prevent harm to human and animal health and to the environment could often be different at the laboratory scale and at a level required for commercialization. Most large-scale applications rely on the modified organism being no more dangerous than the nonmodified host. In instances where the risk assessment of the host organisms indicates a possibility for causing disease or unforeseen adverse effects, physical and/or biological containment may offer solutions. This chapter examined the mechanisms that ensure safety in large-scale applications of GMOs such as GILSP for GM microorganisms, and physical and biological containment appropriate for field release of GM crops and transgenic animals. The chapter also discussed GMPs for the production of medicinal products from biological sources.

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FURTHER READING

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